

09 01

Activity of an acid lipoxxygenase at the surface of sunflower protoplasts**E. BRAIDOT, A. VIANELLO, G. BASSI and F. MACRI'***Cattedre di Fisiologia Vegetale e Biochimica Vegetale, Università degli Studi di Udine, Via Cotonificio 108, I-33100 Udine, Italy*

Sunflower protoplasts exhibited a lipoxxygenase activity inhibited by propyl gallate, SHAM and BHT, and stimulated by the addition of 1.5 mM CaCl_2 , 5 nM H_2O_2 and 1 mM ATP. EGTA or EDTA prevented the Ca^{2+} -stimulated lipoxxygenase activity. Negligible lipoxxygenase was released from protoplasts after 6 h incubation, whereas mechanical disruption of cell integrity, liberating soluble lipoxxygenase, strongly increased such an activity. However, microsomal membranes obtained from hypocotyls still retained a part of this activity which was also recovered in a highly purified plasma membrane preparation.

It is suggested that lipoxxygenase may be localized on the plasmalemma of protoplasts on the basis of the following findings: i) the activity was tightly associated to a purified plasma membrane fraction; ii) exogenously supplied ATP stimulated lipoxxygenase activity; iii) EDTA and EGTA, which similarly to ATP are unable to penetrate into the protoplasts, prevented Ca^{2+} -stimulated activity.

09 02

Study of vacuolar and ubiquitin dependent proteolysis in maize roots during sugar starvation**R. BROUQUISSE, F. JAMES and P. RAYMOND***Station de Physiologie Végétale, INRA, C.R. de Bordeaux, BP 81, 33883 Villenave d'Ornon, France.*

Excised maize (*Zea mays*, L.) root tips were used to monitor the changes in different proteolytic activities, related with vacuolar and ubiquitin-dependent (20S proteasome) proteolysis, during a four-day period of glucose starvation. Autolytic protein degradation was shown to increase throughout starvation (up to 96 h). This rise in autolytic activities was mainly due to the induction of one particular root-starvation-induced-protease (RSIP). Purification, analysis, and immunocytolocalization have shown RSIP to be a 60 kDa monomeric serine-endopeptidase localized in the vacuole of root tissues. Western-blot analysis showed that the maximum RSIP protein level occurs after 72 hours of starvation. On the other hand, purification of 20S proteasome, activity measurements, and western-blot analysis showed that 20S proteasome protein levels and activities increased during 12 to 24 hours of starvation and then decreased. These results suggest that the selective ubiquitin-dependent proteolysis could be involved in the early response to starvation. Subsequently, under prolonged carbon-deprivation, vacuolar proteolysis becomes predominant. It allows cell survival through autophagic processes, which finally lead to cell death.

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09 03

The effect of sucrose, cytokinins, and selected inhibitors of ethylene biosynthesis and action on the longevity of cut *Ixia* flowers

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Cut *ixia* flowers (*Ixia hybrida*), similarly as many other bulb-type flowers, have a very short vase life. However, 24-hours pulsing with sucrose solutions (1-6%) diminished fresh weight decrease and extended their lasting for about one day. Sucrose also accelerated bud opening, although the percentage of flowers developed after harvest was not affected. Higher concentrations were more effective than lower ones.

Conversely, 24-hours treatment with inhibitors of ethylene biosynthesis and/or action did not significantly prolong their life. The ethylene production and EFE activity in different parts of the flower at various developmental stages were also measured. They produced small amounts of ethylene but showed high EFE activity, especially at the bud stage.

09 04

Senescence in conifer needles and changes in endogenous phytohormones

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Conifer trees with needles of different age-classes offer an interesting opportunity to study how the different hormones work together in the regulation of senescence. Here, every needle age-class represents a stage in subsequent changes that finally lead to a hormone status which triggers senescence. We have studied the simultaneous changes in endogenous levels of ethylene (ACC, MACC), abscisic acid and indoleacetic acid (IAA) with increasing needle age in silver fir (*Abies alba* Mill.), taking into account diurnal and seasonal dynamics. We found that contents of IAA at first increased with increasing needle age, but dropped in the eldest needles. The older needles further seemed to lose their ability to form MACC from ACC, a conversion which is important in control of ethylene production. We suppose that elevated internal ethylene levels together with reduced IAA-concentrations trigger senescence development in conifer needles and finally activate the abscission layer at the needle base.

09 05

Parameters to determine drought-stress in tulip**D. CIKANEK, J. M.FRANSEN, H. GUDE AND W. A. KANNEWORFF***Bulb Research Centre, P.O.Box 85, 2160 AB Lisse, The Netherlands*

Before arriving at the flower auction, tulips are often kept dry during several days. However, some tulip cultivars have a better longevity if stored on water. This research aims to find parameters to test under which conditions the tulips were stored previous to trade. Absciscic acid and parameters of oxidative stress, viz. lipoxigenase, superoxide dismutase and lipid peroxidation products were determined.

The absciscic acid level in dry-stored tulips increased severely, while the level in tulips stored on water was only slightly enhanced. No changes in parameters of oxidative stress, however, were observed.

09 06

Change of Phytohormonal Status Leaves in Connection with Hamper of their Senescence**B.I.GULYAEV, D.A.KIRIZIY, A.P.GRIGORTSCHUK, S.V.SAVINSKY, V.V.MORGUN***Institute of Plant Physiology and Genetics, Academy of Sciences, Vasilkovskaya 31/17, 252022 Kiev, Ukraine*

It was shown that the removal of sugar beet apex and shading of maize plant which hamper the senescence of their leaves result in the increase of IAA and decrease of zeatin content in them. The ratio of IAA/zeatin is increased about 2.5 times against the control. In control sugar beet leaves this ratio was 0.95 ± 0.10 , in control maize leaves – 0.26 ± 0.01 . It has been concluded that the change of this ratio reflects the processes which influence the rate of leaf senescence.

09 07

Highly purified soybean plasma membranes exhibit lipoxygenase activityF. MACRI¹, E. BRAIDOT, E. PETRUSSA and A. VIANELLO*Cattedre di Fisiologia Vegetale e Biochimica Vegetale, Università degli Studi di Udine, Via Cotonificio 108, I-33100 Udine (Italy)*

Highly purified soybean plasma membranes exhibit a lipoxygenase activity, with a pH optimum in the acidic (5.5-6.0) range, inhibited by nordihydroguaiaretic acid (NDGA), salicylhydroxamic acid (SHAM) and propyl gallate. It is stimulated by CaCl_2 up to 0.25 mM, H_2O_2 (5 to 10 nM range) and by some nucleotide triphosphates (125 to 1000 nM range) in the following order $\text{ATP} > \text{GTP} = \text{UTP} > \text{CTP}$. The enzyme is not released by treatment of the membranes with 0.05% Brij 58 and is ca. 65% inhibited by the impermeant PCMPs only when the vesicles are permeabilized by 0.01% Triton X-100. Acid lipoxygenase is membrane-bound and may be distinguished from the soluble counterpart, because the latter is not stimulated by nucleotide triphosphates.

The plasma membrane vesicles also show a lipoxygenase, active in the alkaline (9.0-9.5) range, inhibited by NDGA, SHAM and propyl gallate, stimulated by H_2O_2 and less sensitive to calcium stimulation than the acidic one.

09 08

Effect of pollination on ethylene production, ACC content and EFE activity in various parts of petunia /*Petunia hybrida*/ flowers

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Ethylene production, 1-aminocyclopropane-1-carboxylic acid /ACC/ content as well as ethylene forming enzyme /EFE/ activity were detected in various parts of pollinated and unpollinated petunia flowers in four stages of their development and senescence.

The highest amounts of ethylene were formed by pistil, isolated from pollinated flowers and divided into stigma, lower and upper part of the style and ovary. In these parts of gynoecium the high content of ACC as well as high activity of EFE were found. An increase of ACC content and intensified ethylene synthesis in pollinated stigma suggests the participation of this hormone in post-pollination processes in the style. Formation of the abscission layers at the base of the style and corolla, was accompanied by elevated ACC content, increased EFE activity and higher ability of ethylene synthesis. Pollination shortened also the longevity of flowers, both cut and left on the plant.

A great differences in ethylene production observed between corolla and style, suggest a considerable differentiation of these parts of petunia flower in respect to the sensitivity to this hormone.

09 09

Calcium-dependent resistance of fruits during senescence**E.B. MAXIMOVA, N.S. BAJUREANU***Institute of Plant Physiology, Academy of Sciences, Lesnaya 22, 277018 Kishinev, Republic of Moldova*

The structural basis of calcium metabolism in fruits by its visualization and quantitative registration on cell and tissue levels was investigated using electron histochemistry and selective electrodes. The concentration of calcium in pericarp falls into 2 systems. The first one, superficial, participating in the protective-regulatory function is maintained by calcium ions, and the second one, internal, with a reserve function is characterized by lower resistance. Consequently, formation of the property of immobilization as a strategy of fruit life function supply is closely associated with the compartmentalization of calcium localization in the range of 2 of these systems.

09 10

Ethylene and auxin regulation of chilling-induced leaf abscission in *Ixora***S. MEIR, E. YIHYE, Y. REUVENI and S. PHILOSOPH-HADAS***Dept. of Postharvest Science of Fresh Produce, The Volcani Center, Bet Dagan 50250, Israel*

The chilling symptoms of the tropical ixora (*Ixora coccinea*) flowering potted plants are expressed as abscission of leaves 1-3 days following chilling. The degree of leaf abscission, assayed in flowerless potted plants, was positively correlated with the severity of the chilling stress (3.5 and 9°C for 1-3 days). Exposure of ixora plants for 3 days to these chilling temperatures did not affect significantly their ethylene production rates, but resulted in a twofold elevation of 1-aminocyclopropane-1-carboxylic acid (ACC) levels at the abscission zone. However, spraying the plants with 0.2% silver thiosulfate (STS), did not reduce leaf abscission. Hence, it seems that the chilling-induced leaf abscission of ixora plants was apparently not associated with ethylene action. Nevertheless, exposure of plants to exogenous ethylene (3 and 10 ppm) for 3 days enhanced significantly their leaf abscission only when preincubated at low temperatures. Spraying control or ethylene-treated plants with 0.1 mM naphthalene acetic acid (NAA), reduced significantly their chilling-induced and ethylene-enhanced leaf abscission. Results suggest that the chilling-induced leaf abscission of ixora plants, which is associated with alteration of the tissue's sensitivity to ethylene, can be prevented by NAA treatment, which renders the tissue's insensitivity to the hormone.

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09 11

Possible involvement of isocitrate dehydrogenase in senescence**B. NIERI, L. DE BELLIS, P. BIAGI AND A. ALPI***Dipartimento di Biologia delle Piante Agrarie, sez. Fisiologia Vegetale, via Mariscoglio 34, I-56124, Pisa, Italy*

NADP⁺-linked isocitrate dehydrogenase (EC 1.1.1.42) was studied both in germinating and in senescing pumpkin cotyledons. Native page on germinating cotyledons showed that some isocitrate dehydrogenase isozymes are present only in early stages of development, since the isoforms having the lowest mobility tend to fade with the age while the fastest ones become predominant beginning at the 4th day of dark germination and after illumination. A comparative native page performed on both green and senescent cotyledons and on green and senescing leaves of pumpkin showed that the slowest isoforms tend to re-appear in senescence. These data suggest that specific isozymes of NADP⁺-linked isocitrate dehydrogenase are involved both in germination and in senescence, according to an increasing activity of glyoxylate cycle during these phases.

09 12

Abscission - the consequence of specific inter-tissue signalling**D.J. OSBORNE***Open University Oxford Research Unit, Foxcombe Hall, Boars Hill, Oxford OX1 5HR, UK*

Precisely delimited regions of cell separation lead to the shedding of leaves, flowers and fruits. These events occur where a complex of distinct cell types has positionally differentiated early in organ development. Together the cell complex forms a functional abscission zone which remains quiescent until the first signal to separate is perceived. In most plants, but not all, the initiating signal is ethylene and the production of this hormonal signal must be sufficient to outweigh the local auxin levels that maintain the quiescent state. *In vivo*, this condition is attained when ethylene production rises in senescent leaves, fading flowers and ripening fruits. It appears, however, that plants have developed a sophisticated morphological and biochemical organization of the abscission zone which ensures that the cell separation programme shall not proceed to completion in the absence of secondary signals produced and perceived by specific member cells of the complex. Evidence for the plurality of abscission zone target cells will be discussed with reference to leaves and fruits and non-abscinding mutants.

09 13

Inhibition of ripening and ethylene production by application of acetaldehyde vapor

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Exogenous application of acetaldehyde (AA) vapors to various fruit prior to storage caused inhibition of fruit ripening expressed by delay in color development and fruit softening, reduction in decay, and inhibition of ethylene production.

Addition of 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor of ethylene, to citrus, grape, avocado and mango disks that were previously treated with AA vapor showed that AA inhibited ethylene production and ACC oxidase activity. On the other hand, no inhibition was found in ethanol treated fruit disks. CO₂ production increased after addition of ACC in the disks; however, the control disks produced more CO₂ than the AA-treated ones, but less than the ethanol treated disks.

Moreover, the AA treatment inhibited oxidation of the pulp tissue, while the control oxidized and became brown. In the AA-treated avocado the level of total free sulfhydryl group compounds increased, while in the control it remained steady.

09 14

Fatty acids metabolism in peroxisomes of senescent leaves

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Glyoxysomes of germinating pumpkin cotyledons can convert the fatty acids of storage lipids into organic acids by the β -oxidation pathway and the glyoxylate cycle. The fate of acetyl CoA, produced by the β -oxidation, is up to now under discussion for leaf and unspecialized peroxisomes and for peroxisomes from senescent tissues. Since recent studies have demonstrated that during senescence of various organs (leaves, flowers cotyledons) the peroxisomes show novel enzymatic activities of glyoxylate cycle, pumpkin (*Cucurbita* sp.) senescent green cotyledons are investigated in order to demonstrate a direct involvement of these peroxisomes in the β -oxidation pathway and glyoxylate cycle. These peroxisomes can metabolize ¹⁴C-Palmitoyl CoA to ¹⁴C-acetyl CoA by the β -oxidation. Further incubations of peroxisomes with ¹⁴C-acetyl CoA and malic acid indicate that citric acid can be formed and shown by TLC and anion exchange column chromatography. We can suggest that peroxisomes of senescent tissues can utilize fatty acids in order to produce citric acid. Further investigations are in progress to define the ability of peroxisomes to operate the global glyoxylate cycle.

09 15

Association of plum (*Prunus domestica* L.) fruit growth and ripening with ethylene production**H. PLICH***Research Institute of Pomology and Floriculture, 96-100 Skierniewice, Poland*

Changes in the endogenous ethylene production in relation to some ripening parameters in plum fruit cv. Stanley treated with ethephon or ripened naturally were monitored both in pre- and postharvest period. During the last 3 weeks of fruit maturation and ripening on the tree some slow fruit softening and a parallel rise of total soluble solids (TSS) were recorded. But in that period no any increase of ethylene production was observed. A sudden rise of ethylene production started at the picking time and went on thereafter. It was associated with a great acceleration of fruit softening. In that time the level of TSS was increasing too, but at the same rate as before picking. It seems probable that the rise of ethylene production after harvest is correlated with the faster breakdown of plum tissues but scarcely with the regulation of sugar metabolism. The treatment of plum fruit with ethephon about 4 weeks before harvest caused in short time the rise of EFE activity and the earlier increase of ethylene production associated with acceleration of fruit softening and the earlier increasing of TSS. The results show that plums are quite sensitive to ethylene in a long time in advance of being capable to produce it in larger amounts.

09 16

Fatty acid and sterol contents during tulip leaf senescence induced by methyl jasmonate**M. SANIEWSKI*, J. CZAPSKI** and M. HORBOWICZ***Research Institute of Pomology and Floriculture, Pomologiczna 18, 96-100 Skierniewice, Poland***Research Institute of Vegetable Crops, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland***

It has been shown previously that methyl jasmonate (JA-Me) applied in lanolin paste on the bottom surface of intact tulip leaves causes a rapid and intense of its senescence. The aim of this work was to study the effect of JA-Me on free and bound fatty acid and sterols content during tulip leaf senescence.

The main free and bound fatty acids of tulip leaf, in decreasing order of abundance, were linolenic, linoleic, palmitic, oleic and stearic acids. Only the content of free linolenic acid decreased after treatment with JA-Me during visible stage of senescence. β -Sitosterol (highest concentration), campesterol, stigmasterol and cholesterol were identified in tulip leaf. Methyl jasmonate evidently increased the level of β -sitosterol campesterol and stigmasterol induced senescence. It is suggested that the increase in sterol concentrations under the influence of methyl jasmonate induced changes in membrane fluidity and permeability, and these findings will be discussed.

09 17

Changes of the growth-regulating processes in aging segments of maize coleoptiles

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Aged segments (24 hs after exision) had 3 times smaller growth rate and growth response to the auxin . At the same time the activities of H⁺-pump and ATP-ase in plasma membrane increased. The aging did not affect auxin-induced positivation of tissue electric potential related to proton secretion. But cell walls of aged segments only slightly extended in acid buffers and "acid growth" of the segments was very small. Transport of several proteins to the cell walls and incubation medium changed. The reduced efflux of one protein fraction with peroxidase activity could cause alteration in cell wall rigidity because the water solution of these proteins increased segments extension growth. We suppose that the main cause of growth loss of aging coleoptile segments is the decrease of cell wall extensibility whereas the plasma membrane processes of auxin-dependent elongation remain unaltered.

09 18

Senescence, enzyme synthesis and metabolism

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Senescence of cells, organs and whole plants is a normal part of plant development. It is genetically programmed, as shown by mutants with altered senescence and by studies of gene expression during senescence. Synthesis of new proteins and activation of metabolic pathways occurs. The metabolic activities of senescing tissues utilise cell constituents for energy production and for use by other parts of the plant. We have studied the synthesis of enzymes of the glyoxylate cycle and gluconeogenesis during organ senescence. Results indicate that the glyoxylate cycle and gluconeogenesis are active in senescence, presumably making use of products of breakdown of cell constituents to synthesise sucrose for export from the senescent organ. However in detached organs induced to artificially 'senesce', gluconeogenesis may not be activated, but instead the glyoxylate cycle may serve an anapleurotic role to support the citric acid cycle in mitochondria, and amino acid metabolism. Investigating gene expression in senescence gives us clues about metabolic activities which are taking place, and provides an opportunity to genetically manipulate the senescence process, and hence plant growth and development.

09 19

The telomeres of *Arabidopsis thaliana*: length and protein structure

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In all eukaryotic organisms investigated so far the telomeres are conserved structures which are essential for the stability and complete replication of the chromosomes. In animal cell lines, the shortening of the telomeres seems to be associated with senescence. Here, the lengths of the *Arabidopsis thaliana* telomeres were determined in different developmental stages, but it seems that the bulk of telomeres do not shorten during whole plant cell senescence.

Proteins specifically binding to telomeres of *Arabidopsis thaliana* were characterized by gel mobility shift assays with synthetic oligonucleotides consisting of four 7-bp telomeric repeats of *Arabidopsis* (TTTAGGG) and crude nuclear protein extracts of *Arabidopsis* leaves. These DNA-protein binding studies revealed that the binding-affinity of these telomere-binding proteins to the G-rich single strand as well as to the double-stranded telomeric DNA is much higher than to the C-rich single strand. The molecular weight of the proteins was identified by SDS-PAGE to be 65-67 kDa. The isoelectric points were determined to be 5.0, 4.85 and 4.7, respectively. An RNA component probably serving as a template for reverse transcription of a plant telomerase could not be detected in the DNA-protein complexes with the telomeric DNA, neither was the DNA-protein interaction RNase-sensitive.