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Conference report
Plant Biology '96
Annual meeting of the American Society of Plant Physiologists
San Antonio, Texas
July 27-July 31, 1996

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Summary
The Annual meeting of the American Society of Plant Physiologists (ASPP) 1996 was held in San Antonio, Texas. Around 900 people joined the meeting. The program of the conference included 5 symposia, 8 minisymposia, 19 oral sessions and over 800 posters covering all current topics of plant biology.
Sessions and individual presentations or posters of interest for ATO-members will be briefly mentioned in this report. For detailed information there is a book of abstracts available at the rooms of Koos Oosterhaven (2-06), Gerard Rouwendal (2-18) or in the library.

Program of the conference
Titles of the sessions are listed below and some remarks are given about interesting topics. For details please check the **Abstracts.**

- *News from the frontiers of the cytoskeleton*

Susan Wick (Univ. of Minnesota) is the major scientist in the field of the cytoskeleton research. The cytoskeleton is composed of networks of actin microfilaments, micro tubules, intermediate filaments and their associated proteins. It participates in diverse developmental and physiological processes, such as mitosis, cytokinesis, cytoplasmic streaming, intracellular transport, positioning of organelles and macromolecules, cellular polarization, tip growth, tracheary element differentiation, morphogenetic divisions, stomatal guard cell movements, and intercellular transport of macromolecules through plasmodesmata. Relevant genes, such as a- and b-tubulin, have been cloned and accumulation of specific isoforms in various parts of - *Arabidopsis* was reported. Anti-sense plants have been made and evaluated.
The role of actin-cytoskeleton expression and organization during root-nodule development was discussed by Sanchez (Univ. of Mexico). The regulation of actin organization by small actin-binding proteins (ABP's) was reviewed by Steiger (Univ. of Lafayette). ABP's are about 14 kDa and 10 genes (70-90% homology) have been described so far. One such protein is profilin in maize. The cDNA is able to rescue a mutant in Dictostelium. Profilin (2-6% of total protein) binds to/ is a ligand for actin, phosphoinotoside lipids (PIP) and poly-L-proline. One profilin can bind up to 10 PIP molecules and can play a role in signal transduction. After injection of profilin (in cells) the nucleus loses its original shape and position and therefor causes a nuclear displacement. This phenomenon is used for an assay to quantify the effect of several chemicals/hormones on the profilin-metabolism and action.

- *Molecular biology, biochemistry and physiology of plant P450's*

The plant P450's consist of 26 superfamilies (family: >40% homology; subfamily:

>55%; loci: 55-97%; allelic variance: >97% homology) and are highly inducible. They are membrane bound, act together with a reductase (use of molecular O_2 and -NADPH), are slow, and are assumed to be the key-rate enzymes.
Functions of the cytochrome P450 monoxygenases are: oxidative reactions, reduction reactions in synthesis of a lot of secundairy compounds e.g. furanocoumarins, monoterpenes, anthocyanins; detoxification-reaction of xenobiotics.
Research concerns a wide variety of subjects:
1. plant-insect interface (synthesis of toxic compounds and induction of detoxification reactions)
2. environmental effectors and substrates
3. herbicide detoxification (O'Keefe, DuPont Company). Perhaps the P450 enzymes may be used for detoxification/conversion of other undesirable compounds in plants.
4. flower colour; blue colour (Florigene)
5. selection markers;
6. drug biosynthesis; 25% of modern drugs are derived from alkaloids!
7. expression of these enzyme systems in yeasts or bacteria
8. In the Werck-Reichhart lab in Strasbourg, selective induction of P450 genes by different pollutants is used for monitoring environmental pollution. This might also be useful for screening food for example.
9. regulation; by UV, wounding, chemicals/elicitors, osmotic shock, etc.
In parsley, the regulation of the P450 genes encoding PAL and C4H is at the level of the mRNA

- *Advanced techniques in plant biology*

Expression of GFP in transgenic *Arabidopsis* was shown to be dependent on modification of the coding region as was also done at ATO-DLO (Haseloff). New variants of the gene were made to overcome GFP toxicity and to enhance brightness. In addition, the protein was targeted to the endoplasmic reticulum which also served to increase GFP fluorescence. A spectacular video was shown of confocal images of transgenic *Arabidopsis* roots in which the central cylinder was marked by GFP fluorescence; a complete 3D analysis of GFP expression in a specific tissue could thus be performed.

Developmental processes

* *Regulation of senesence in carnation petals by the ovary.* It was demonstrated and concluded that ethylene nor ACC were the senesence-inducing compounds (Benson, Univ. of Santa Clara, CA). It was hypothesized that the immature ovary releases a signal that induces senesence.
* *Somatic Embryogenesis* (SE) is reduced by ethylene. SE in transgenic cells, expressing mammalian ODC (ornithine decarboxylase) is related to an increased

polyamine metabolism: putrescine increased, spermine and spermidine was unaltered. The transgenics showed an enhanced SE but produced little ethylene. Therefor Minocha (Univ. of New Hampshire) concluded that PA-metabolism is able to take SAM from the ethylene pathway.

* *Banana fruit ripening* is studied by the Boyce Thompson Institute for Plant Research, Ithaca (by Clendennen). The ultimate goal is to find tissue and stage specific promotors for the synthesis of vaccins (like hepatitis B surface antigen or *E. coli* enterotoxin B subunit) in plants. Both of these genes had be resynthesized to adapt their codon usage to the plant environment for optimal gene expression (Mason).

Using a differential screening 4 classes of genes (mRNA-screening) could be distinguished:

1. starch related (GBSS, SS)
2. PR-related (β-1,3 glucanase, chitinase, endochitinase, thaumatine)
3.MT-transcripts (Metallo-Thioneine) => anti-oxidant during high respiratory activity (climacteric for instance)
4. lectins

* Coupe (Pennsylvania state Univ.) investigates the *ethylene response* during tomato fruit ripening. E4 and E8 are two transcripts that occur after ethylene induction. The 5' flanking regions are studied in relation to the E4/E8 binding protein (BP), that interacts with the 5' flanking regions of both genes. On the basis of the sequence of the 51 kDa BP, the protein has a zinc finger structure. The authors suggest that E4/E8 BP participates in the complex regulation of E4 and E8 transcription.

* Lewandowski (Univ. of Florida) studied the premature abscission of citrus fruits. in citrus 6 abscission zons could be identified and ethylene increases abscission by inducing specific cellulases and hydrolases. Abscission cellulases have been identified in several species. Two cellulases were cloned from citrus. Hydrolase activity and PG activity could be related to the fruit removal force. The higher these enzymatic activities, the lower the fruit removal force (the force needed to remove the fruit from the tree).

- *Signal transduction*

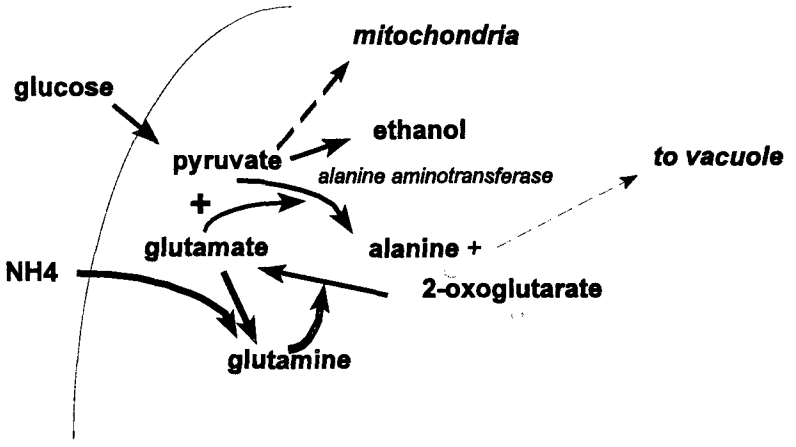
*Muschak (group Willmitzer, Max-Planck Institut) reported the screening of antisense FBP-ase potato plants (fructose-1,6-biphosphate phosphatase). The plants expressed diffrent levels of the chloroplastic isoform of FBP-ase. They showed reduced levels of ABA in the leaves. the authors suggest a possible feed back mechanism from the photosynthetic performance to the stomatal behaviour through ABA.

*Reid and Donovan (Univ. Massachusetts). Polygalactorunase (PG) activity increases just before flower opening, whereas cellulase drops sharply before

opening. The pattern of cellulase activity is consistent with a role in lacuna formation and wall thinning. The antioxidant sodium benzoate was found to be a very effective agent in delaying flower senescence.

- *Interactions of C and N metabolism*

Pretreatment of maize seedlings in low oxygen (3%) improves their root viability in subsequent anoxia. Xia and Roberts (Univ. of California) study the metabolic regulation of these processes. The predominant compound during hypoxia (3% O2) is alanine. Alanine accumulates in the vacuole. The regulation is as illustrated in the scheme:



Alanine accumulation during hypoxia is largely irreversible after reoxygenation, suggesting transport of alanine into the vacuole, sequestering it away from cytosolic and mitochondrial enzymes. They also found that the cytosolic concentration of pyruvate increased during hypoxia. Labeling seedlings with C13-glucose, a much higher concentration of C13-alanine was observed, compared to normoxic or anoxic tissue. This suggests a role for the malic enzyme in supplying pyruvate for alanine synthesis during acclimation.

- *Lipids and related molecules*

*Nunberg (Texas A&M). A delta6-desaturase gene was cloned from borage and transformed into tobacco. The resulting transgenics produced high levels of gamma linolenic acid (GLA) in their leaves (up to 20% of C18 fatty acids).

- *Plant microbe interactions*

* Jackson (Univ. of California). During infection, viruses must replicate quickly in host cells and subvert their metabolic processes to mediate productive replication and spread throughout the plant. Activities involved are:
1. uncoating of viral genome
2. early and late gene expression
3. genome amplification
4. progeny virus assembly.

In addition, the viruses need to overcome both active and passive host defense responses. Viruses developed an array of techniques to direct host metabolsim in favor of virus reproduction, such as diverse mRNA translation techniques, cytoplasmic localization of virus encoded proteins, association of viral replicase proteins with host-factors to form virus specific RNA-dependent RNA-polymerase, and so on.

* The role of bacterial secreted proteins and their secretion pathways in the development of plant-pathogen interactions was discussed by Collmer (Cornell University, Ithaca). Several bacteria elicit a hypersensitive response (HR) in plants. They can do that by secreting so-called Hrp proteins (harpin proteins) in the host tissue. Harpins elicit an HR in tobacco, they are secreted in tissue culture, are heat stable, glycin rich and lack cystein, and there are mutant fenotypes available. Therefore they form an interesting subject to study the role of HR and Avirulence (Avr) genes.

* The studies of Briggs (Pioneer Hi-bred International Inc. Johnston) focus on HC-toxin and the detoxification of this cyclo tetrapeptide, produced by several microbes (a.o. *Cochliobolus carbonum* on maize). HC-toxin can be detoxified by HC-reductase (HCTR) produced by maize. HC-toxin itself inhibits histone deacetylase. Inhibition of histone deacetylase blocks the induction of defense gene transcription, thereby causing susceptibility. Histone proteins need to be acetylated for an appropriate activity. Deacetylation causes gene activation.

If a fungus attacks a plant, the plant reacts with a typical response: salicylic acid and PR proteins accumulation. However, if also the HC-toxin is applied to these plants, the typical defense response is blocked since transcription is blocked, due to inhibition of the histone deacetylase.

- *Transgenics and biotechnology*

* Ebinuma. At this meeting, the biotechnology lab of Nippon Paper introduced the MAT vector system, which is a novel plant transformation technique featuring the removal of the 'morphological abnormality inducing' marker following the transformation. The most important advantage of such a system from the point of view of the biotechnology companies lies in the possibility to use the same marker over and over again. In addition, transgenic crops may meet with less public anxieties

when antibiotic or herbicide resistances would no longer be required for plant transformation.

* Duwenig (Willmitzer lab, Germany). Transgenic potato plants were made with antisense constructs containing the genes for cytosolic (Pho2) and plastidic starch phosphorylase (Pho1). The most important finding may be that antisense Pho2 plants produce more tubers per plant with a higher fresh weight per tuber. Sugar accumulation in cold stored tubers was not affected.

* De Rocher (MSU-DOE East Lansing). The (posttranscriptional) mRNA instability of Bt. toxin genes expressed in plants could be greatly reduced by adapting codon usage. But mRNA instability alone does not account for low expression of the toxin genes in plants. Understanding of these factors will improve the application of heterologous genes in plants.

* Webb (Texas Tech Univ.). Tobacco was transformed with constructs containing pea cytosolic ascorbate peroxidase (APX) targeted to the cytosol or to the plastid. Both types of transformants showed a significant increase in protection from the superoxide-generating herbicide methyl-viologen (MV). Antisense inhibition of APX was impossible probably due to selection against it during transformation.

* Payton (Texas Tech Univ.). Tobacco was transformed with sense constructs containing the *Arabidopsis* glutathione reductase (GR) gene. GR levels in the resulting transgenics were greatly induced, but the highest expressors were only slightly more resistant to MV treatment.

* Hedden (IACR-Long Ashton Res. St. Bristol). *Arabidopsis* was transformed with sense and antisense constructs containing GA 20 genes involved in gibberellin synthesis. Antisense constructs had no clear phenotypic effect. Overexpression of the GA 20 gene caused earlier bolting and increased final stem length indicating that this step in gibberellin synthesis may be rate-limiting.

- *Mitochondria and respiration*

* Pyruvate synthesis via malic enzyme (ME) was measured *in vivo* in maize root tips during normoxic and hypoxic conditions by Edwards (California Univ Riverside). The method used consisted of a coupled technique of GC/MS and ^{13}C NMR. 1-^{13}C-glucose was fed to root tips. Incorporation of ^{13}C into C1, C2 and C3 alanine is due to ME, whereas ^{13}C in C3-alanine is due to Pyruvate Kinase (PK). GC/MS measurement of absolute ^{13}C incorporation and NMR measurements of relative isotopic labeling of individual carbons, indicated that ME makes a minor contribution to respiration *in vivo*. In contrast, ME increases dramatically when the root tips were exposed to a low oxygen (3%) environment. This activity funnels C and N from aspartate, asparagine and malate, to pyruvate and alanine.

* The pyruvate dehydrogenase complex converts pyruvate (and NADH) into acetyl

CoA and NAD^+. It is an irreversible reaction and therefore believed to play a regulatory role in carbon metabolism. PDC is present in mitochondria and in the plastids and the complex consists of several proteins (6 mDa in total; 4 catalytical subunits, and two regulatory enzymes which total over 200 polypeptides in each complex). Esssential cofactors are pyruvate, NAD^+, TPP, CoA and $MgCl_2$. The complex can be purified from several plant sources. Intensive studies were undertaken into the developmental regulation of *Pisum sativum* PDC. Furthermore from a C3-plant (maize) the complex had not been characterized. The group of Randall (Missouri University, Colombia) presented 3 papers about the characterization of the complex using antibodies raised against several subunits of the complex, and probes for Northern blotting.
Rapid regulation by light is supposed to be due to phosphorylation/ dephosphorylation reactions. The 43 kD E1α-subunit of mtPDC was shown to be 5-fold higher phosphorylated in the light than in the dark and therefore accounting for the regulatory role of light.

* Ribas-Carbo (Duke University, Durham) studies the effect of light starvation in the electron-partitioning between the cytochrome and alternative pathway in soybean cotyledons. He used an oxygen isotope fractionation technique in order to determine the flux of electrons in either the alternative or the cytochtrome pathway. Pyruvate enters the pathway at the level of the ubiquinone. Via cytochrome ATP is produced. Via the alternative pathway only heat is produced. Prior to treatment, the cytochrome and alternative pathways share electrons almost equally (53% cyto). After 24 h darkness, electron partitioning through the alternative pathway decreses till 35%, and continues to decrease till 10% of the total electron flux after 58h of complete darkness. However, total respiration rates was similar in treated and control plants. Since soluble sugars and starch decreased within 12h of darkness a clear relationship was demonstrated between the activity of the alternative pathway and the concentration of sugars and starch.

- *Plant developmental genetics*

The regulation of developmental processes in Arabidopsis is a main research area of several groups (a.o. New York Univ., Univ. of Madison). Some mutants have been isolated: Scarecrow (SCR), Shootmeristemless (STM), Short-root, Leafy, showing developmetal abnormalities. The protein from the Scarecrow-gene may act as a transcription factor regulating the root radial organization. Mutants do not have an asymetric cell division of the cortex/endodermis initial cell, early in development. Asymetric cell division is necesary in order to generate the cell lineages of the root. The expression of Scrarecrow in tissues was determined.
STM mutants do not have shoots. STM is expressed in the apical shoot meristem

and inhibits cell division of the adjacent cells whereas it stimulated cell division of the shoot meristem cells itself. So, STM determins meristem fate depending on its cellular localization.

- *Advances in the study of natural products*

* Chapell (Univ. of Kentucky) produced HMGR transgenes of tobacco using hamster cytosolic HMGR. His group is focused on the biosynthesis of (valuable) monoterpenes, sesquiterpenes, etc. and therefore has cloned several genes involed in the production of these compounds. HMGR-Transgenics showed 5-fold stimulation of HMGR and the sterol content increased from 0.2% till 1.1%. However, the transgenics did not overproduce sesquiterpenes. Furthermore, the plants were in bad shape!
Sesquiterpene cyclase was also cloned and several genetically engineered constructs (differing in their catalytic site) were used in structure-function relationship studies. They showed that depending of the construct (and subsequent protein) it was possible to produce 100% end product A or 100% product B and almost every intermediate amount of A and B. Using these techniques is must be possible to engineer the sesquiterpene pathway in any direction.
* Gershenzon (from the lab of Croteau, WA state Univ.) showed old news on the synthesis of peppermint (menthol and menthon) in *Mentha* species. Geranyl diphosphate is cyclised by Limonene Synthase (LS) which has been recently cloned. Monoterpene biosynthesis is under tight control. The rate of biosynthesis paralleled LS activity, suggesting the importance of LS in controlling the flux through the monoterpene pathway. Using immunocytochemical methods he showed that LS is confined solely to the leucoplasts of the secretory cell. Directing LS into plastids (in pea chloroplasts) with the signal peptide, resulted in a correct processing of the preprotein in a native protein of the predicted size. This demonstrates that plastids can be a major site of isoprenoid biosynthesis. In plastids: monoterpenes, diterpenes, phytol, carotenoids. In the cytosol: sesquiterpenes, sterol, triterpenes, dolichol. In Golgi: plastoquinone and ubiquinones.

Contacts and spin-off

1. An appointment was made with Prof. Bach (Strassbourg Université) to join an EU-project within the Biotech-programme about the biosynthesis, action and toxicity of isoprenoid compounds. This project has now been submitted to the EU.
2. Dr. Jeffrey C. Suttle, a potato researcher from the USDA, Agricultural Services, was contacted about their research on tuber dormancy.
3. Dr. H. Ebinuma was contacted about possibilities to test the MAT vector system. Meanwhile, this request has been granted and a draft agreement was sent to us.