BMC Plant Biology

BioMed Central

Volume 5 Suppl 1, 2005
Meeting abstracts

Cell Biology of Nitric Oxide and Cell Death in Plants

Yalta, Ukraine
8–11 September 2004

Published: 31 May 2005

S1

Influence of UV-radiation on the photosynthesis and photosynthetic carbon metabolism in high mountainous plants

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BMC Plant Biology 2005, **5(Suppl 1):**S1

The influence of UV-radiation on the $^{14}CO_2$ assimilation rate of three high mountainous plants (*Heracleum lehmannianum* Bunge, *Prangos pabularia* Lind L. and *Lathyrus mulkak* Lipsky) with different photosynthetic intensity and directivity of photosynthetic carbon metabolism was studied. The investigation was carried out in the Biological Station of The Institute of Plant Physiology and Genetics (Academy of Sciences of Republic of Tajikistan), located on an austral decline (2350 m above sea level) of the Hissar valley (Tajikistan). It is proved, that plants show different responses to dissecting away of UV-rays. Almost tenfold fall in the intensity of $^{14}CO_2$ fixation (during 30 sec) is noted for *H. lehmannianum*; in *P. pabularia* there was twofold fall in depression, and in *L. mulkak* the dissecting away of UV-rays resulted in the minor rising of the photosynthetic rate. Under both film dropping UV-rays and in open area, we have not revealed essential differences in the $^{14}CO_2$ assimilation rate in all three plants.

The experiments were carried out in three variants:

1. Control – open place.
2. The plants were covered with polyethylene film.
3. The plants were covered with polyethylene film enriched with 2-oxi-4-alcoxibenzophenol (0.65%), cutting off the UV part of solar spectrum.

The study of ^{14}C incorporation into the products of photosynthesis has manifested essential differences between investigated plants. From the control experiment, using the *Heracleum* leaves, more than 65% of a label was included into the intermediates of the Calvin cycle (among them about 20% into PGA). The dissecting away of UV-radiation resulted in a depression of ^{14}C incorporation into PGA and PES. The key products, which, at 30 sec exposure, concentrated the most part of label were sugars, predominantly sucrose. Intermediates of the glycolic pathway concentrated a small part of ^{14}C (22%). The depressing of CO_2 photosynthetic assimilation in *Prangos* was accompanied by a sharp slump of ^{14}C incorporation into intermediates of the Calvin cycle. The label was found out in sugars predominantly and in intermediates of the glycolic pathway. In control plants we detected an increase of ^{14}C lobe in monosaccharides. The content of label in intermediates of glycolic pathway decreased. In *Lathyrus* leaves under investigation we revealed intensive label incorporation into intermediates of the Calvin cycle and its decrease in the metabolites of the glycolic pathway. We found minor quantities of ^{14}C contents in sugars and PEP-products. It is supposed that the UV-rays influence the activity of RUBISCO and other enzymes of the Calvin cycle. The mechanisms of UV-rays influence on carboxylation system and the possibility of their regulatory role in high mountainous plants are under discussion.

S2

Changes in a pattern of HMW-DNA fragmentation accompany differentiation and ageing of plant cells

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BMC Plant Biology 2005, **5(Suppl 1):**S2

Nuclear DNA is arranged into loop domains by periodical attachment of chromatin fibers to nuclear matrix at highest level of chromatin compactisation. Chromatin loops represent the basic structural components of higher order chromatin folding, which are maintained during cell cycle and in differentiated cells. An increasing number of evidences suggests strong relationship between chromatin structure compactisation and functional activity of cell nucleus.

We showed previously that regular DNA cleavage into high molecular weight (HMW) DNA fragments can be induced in intact nuclei, resulting in formation of ~300 kb and 50–100 kb fragments. The patterns of induced cleavage were essentially similar both in intact and high-salt-extracted nuclei suggesting that HMW-DNA fragments represent DNA loop domains (or their associates). Biochemical properties of induced HMW-DNA cleavage suggest involvement of matrix-associated topoisomerase II in excision of DNA loop domains.

We demonstrated further that patterns of HMW-DNA cleavage are distinct in plant tissues differed on proliferative activity and differentiation status. The least fragmentation was found in the embryos of dry quiescent seeds, whereas the induction of growth and development was accompanied by increase in HMW-DNA fragmentation. The study of cell nuclei during natural and accelerated ageing of rye seeds demonstrated that loss of germination capacity was associated with decreased excision of chromatin loop domains.

Taken together, our data demonstrate that changes in physiological status of plant tissues are accompanied with the changes in patterns of HMW-DNA fragmentation. The HMW-DNA fragments are generated by the scaffold-associated topo II-like endonuclease activity that cleaves nuclear DNA probably at the positions of its attachment to nuclear scaffold. We hypothesize that HMW-DNA cleavage activity is related to some DNA metabolic processes in cell nuclei and is necessary for maintaining active physiological status of plant tissues.

S3
UV-A induced fluorescence images in sun and shade leaves

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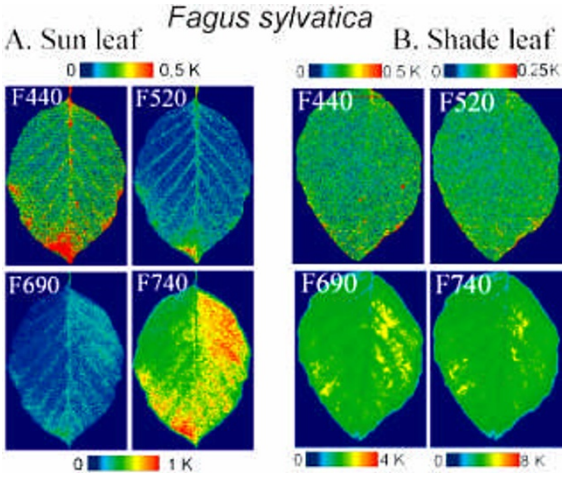
BMC Plant Biology 2005, **5(Suppl 1):**S3

Background: At their natural environment plants are exposed to high fluxes of photosynthetically active and UV radiation. The increased synthesis of UV-absorbing compounds, such as flavonoids and other polyphenols, are accepted as a plant response to increased UV radiation. The increase of leaf flavonoid content can be a protection against the harmful effect of UV-B radiation as they function as antioxidant and UV filters in plants. Differences between sun and shade leaves (adapted to high light and low light respectively) of some tree species are analysed, taking in consideration differences on the UV-A absorption via fluorescence imaging of leaves.
Materials and methods: Leaves of beech (*Fagus sylvatica* L.), maple (*Platanus hybrida* L.) and poplar (*Populus nigra* L.) were analysed. The Karlsruhe flash-lamp fluorescence imaging system (FL-FIS) was used to measure images at the fluorescence bands blue, green, red and far-red. The decline of chlorophyll fluorescence from Fp to the steady-state Fs was imaged too [1].
Results: Shade leaves exhibited a much higher chlorophyll fluorescence yield than sun leaves. Also differences in the distribution of the fluorescence over the whole leaf were detected which are due to the lower chlorophyll content of shade leaves and a lower reabsorption of the emitted F690 band. Shade leaves are characterized by lower values of the fluorescence ratios blue/red (F440/F690) and blue/far-red (F440/F740) than sun leaves. Both ratios are early indicators of stress to the photosynthetic apparatus. The fluorescence ratio red/far-red expressed lower values in sun leaves and is an indicator of their higher chlorophyll content (curvilinear inverse correlation). The R_{Fd}-images (R_{Fd} = (Fp-Fs)/Fs) as indicators of photosynthetic activity allow to evaluate differences in photosynthetic quantum conversion.
Conclusion: The observed differences in the fluorescence images and chlorophyll fluorescence images allow to characterize the different properties of sun and shade leaves related to their adaptation to high light and high UV-radiation or low light and low UV-radiation.

Figure 1 (abstract S3)

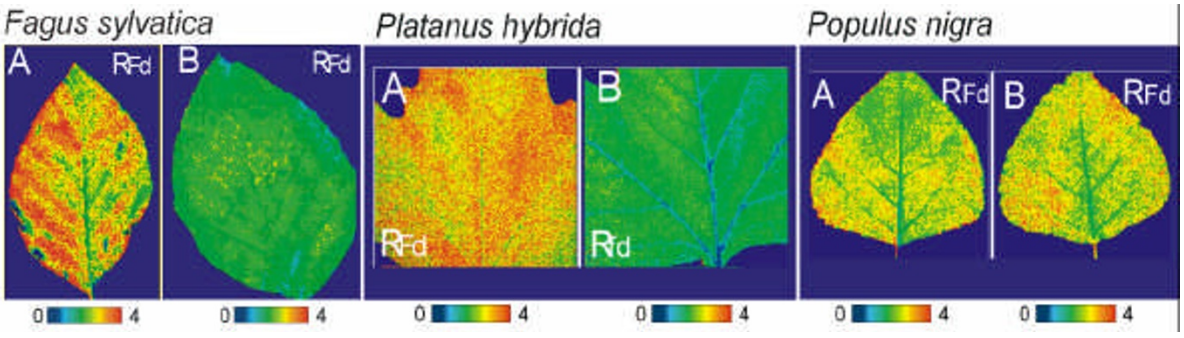


UV-flash lamp induced fluorescence images of the blue (F440), green (F520), the red (F690) and far-red fluorescence (F740) of the sun (**A**) and shade (**B**) beech leaf.

References

1. Lichtenthaler HK and Babani F: **Detection of photosynthetic activity and water stress by imaging the red chlorophyll fluorescence.** *Plant Physiol Biochem* 2000, **38:**889–895.

Figure 2 (abstract S3)



Images of the red chlorophyll fluorescence decrease ratio of a sun (A) and shade (B) leaf of beech, maple and poplar.

S4
Effect of UV-B radiation on some cereals

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BMC Plant Biology 2005, **5(Suppl 1):**S4

An influence of low and high doses of UV-B radiation (280–320 nm from special luminescent lamp TL 20 W/12 RS) on cereals (barley, oat and winter wheat) was investigated.

Plants were cultivated in a greenhouse, and then were displayed under a lamp in a bushing out phase during 4, 8, 12, 24 and 72 h of continuous irradiation on the distance 20 cm. The flux of UV-B irradiation under these conditions was 5.7 wt/m^2. Doses which received plants was 19.6 kal/cm^2, 39.2 kal/cm^2, 58.8 kal/cm^2, 117.6 kal/cm^2 and 352.8 kal/cm^2 respectively. Characteristics of plant response to irradiation were: changes in pigment systems (chlorophylls and carotenoids), changes in the content of phenolic substances, and changes of peroxidase activity also.

It was shown that barley is the most sensitive and winter wheat is the most tolerant to UV-B. However, high doses of radiation caused strong growth inhibition during vegetation period and biomass loss in winter wheat, too. Significant reduction of content of main photosynthetic pigments (chlorophylls *a* and *b*) as well as carotenoids in leaves of tested plants as a result of intensive exposure to UV-B irradiation was established. An increasing of carotenoids content in winter wheat at UV-B irradiation can be considered as adaptive and protective reaction preventing from photodestruction of chlorophylls. An elevation of carotenoids in barley during irradiation in contrast to winter wheat was not observed, that testifies to absence protective and adaptive reaction of the given type in this plant species.

Comparison the data on the contents of phenolic substances in leaves of summer barley with winter wheat and oat at UV-B radiation has shown that the amount of these substances in plants of winter wheat increased weakly in comparison with oat and especially in comparison with summer barley. It allows to assume that increased synthesis of phenolic substances during exposure to UV-B radiation with dose 58.8 kal/cm^2 (12 h of irradiation) and especially with dose 117.6 kal/cm^2 (24 h of irradiation) testifies to stressful condition for barley plants. Respectively, increasing of phenolic substances content can be considered as one of protective reactions in UV-sensitive plants. Increased peroxidase activity under the influence of low UV-B doses testifies to stressful condition and protective processes in tested plant (barley). The reduction of peroxidase activity immediately after exposure to excessively high dose UV-B radiation (225.2 kal/cm^2) during 48 h is the evidence of already non-irreversible damages in test plants.

S5
Ambient levels of UV radiation

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BMC Plant Biology 2005, **5(Suppl 1):**S5

The levels of solar ultraviolet (UV) radiation at the Earth's surface vary widely, depending on the atmospheric and environmental conditions of the observation site. Under clear sky conditions, the most important parameters are solar zenith angle, total ozone content, amount and type of aerosols, altitude above sea level and albedo of the ground [1]. In addition, attenuation and in special cases amplification by clouds has to be considered. Based on detailed measurements under a large number of different conditions, the effects of the individual parameters on global UV irradiance are quantified. Most of these effects depend strongly on wavelength and many of the effects are most pronounced in the erythemally weighted UV-range.

The effects of the most important parameters are: 1% decrease in ozone results in an increase of erythemally weighted UV irradiance of about 1.1% [2]; aerosols can attenuate erythemally weighted UV irradiance by up to 30%, but this depends strongly on amount and type of aerosols [3]; the increase of erythemally weighted UV irradiance with an increase in altitude of 1000 m amounts between 15% and 25%, for UVA-radiation this increase is about half that much [4]; an increase of ground albedo by 0.5 will increase erythemally weighted irradiance by about 17%, UVA irradiance by about 10% [5]; a complete cloud cover attenuates UV irradiance about 40% less than total irradiance [6].

Long-term changes of solar UV radiation are a complex combination of various effects, where stratospheric ozone depletion, increase of tropospheric ozone levels and effects of global climate change work together. Therefore predictions for future levels of UV radiation are limited to the assumptions of specific scenarios. Future measurements will be necessary to monitor any changes on a local and on a global scale.

References

1. Blumthaler M and Webb A: **UVR climatology.** *UV effects in Aquatic Organisms and Ecosystems. Comprehensive Series in Photochemistry and Photobiology* The Royal Society of Photochemistry, Cambridge: Helbling EW, Zagarese H 2003, **1:**21–58.
2. Blumthaler M, Salzgeber M and Ambach W: **Ozone and ultraviolet-B irradiances: experimental determination of the radiation amplification factor.** *Photochem Photobiol* 1995, **61:**159–162.
3. Kylling A, Bais AF, Blumthaler M, Schreder J and Zerefos CS: **The effect of aerosols on solar UV irradiances during the PAUR campaign.** *J Geophys Res* 1998, **103:**26051–26060.
4. Blumthaler M, Webb AR, Seckmeyer G, Bais AF, Huber M and Mayer B: **Simultaneous Spectroradiometry: A Study of Solar UV Irradiance at Two Altitudes.** *Geophys Res Let* 1994, **21:**2805–2808.
5. Gröbner J, Albold A, Blumthaler M, Cabot T, De la Casinieri A, Lenoble J, Martin T, Masserot D, Müller M and Philipona R: **Variability of spectral solar ultraviolet irradiance in an Alpine environment.** *J Geophys Res* 2000, **105:**26991–27003.
6. Blumthaler M, Ambach W and Salzgeber M: **Effects of cloudiness on global and diffuse UV irradiance in a high-mountain area.** *Theor Appl Climatol* 1994, **50:**23–30.

S6
Regulation of programmed cell death in plant embryogenesis

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BMC Plant Biology 2005, **5(Suppl 1):**S6

As plants grow they not only form new tissues and structures using highly coordinated cell-division and cell-differentiation

programs but also continuously kill many of their own cells through activation of programmed cell death (PCD). The earliest functions of PCD in plant life are fulfilled during embryogenesis. Here PCD governs two major developmental processes. First is the elimination of a transient embryonic structure – suspensor, which functions at early stages of embryo development as a conduit of nutrients and growth factors, but is not required at later stages [1]. Another function of embryonic PCD applies to embryo abortion, which is not only a response to stress or mutagens, but also a normal feature of those plant species, which produce polyembryonic seeds. In the latter case competition among multiple embryos for survival often induces PCD resulting in the elimination of all but one embryo in a seed [2]. At the demise, both suspensor and entire embryos display a gradient of successive stages of PCD along apical-to-basal axis [2, 3]. This PCD implicates active role of autophagy in complete removal of cell protoplast. Autophagosomes are formed via Golgi and proplastids [1]. Autophagocytosis depends on the dynamic reorganization of the cytoskeleton. Microtubule network is disrupted early in PCD pathway. F-actin is gradually reorganised into thick longitudinal cables and is present till the vacuole collapse and fragmentation of nuclear DNA [1, 3]. Type II metacapase is critically involved in the regulation of the cell death pathway, which is essential for normal embryo development. Metacaspase gene silencing results in the suppression of cell death and failure of embryonic pattern formation [4].

References

1. Filonova LH, Bozhkov PV, Brukhin VB, Daniel G, Zhivotovsky B and von Arnold S: **Two waves of programmed cell death occur during formation and development of somatic embryos in the gymnosperm, Norway spruce.** *J Cell Sci* 2000, **113:**4399–4411.
2. Filonova LH, von Arnold S, Daniel G and Bozhkov PV: **Programmed cell death eliminates all but one embryo in a polyembryonic plant seed.** *Cell Death Differ* 2002, **9:**1057–1062.
3. Smertenko AP, Bozhkov PV, Filonova LH, von Arnold S and Hussey PJ: **Reorganisation of the cytoskeleton during developmental programmed cell death in *Picea abies* embryos.** *Plant J* 2003, **33:**813–824.
4. Suarez MF, Filonova LH, Smertenko A, Savenkov EI, Clapham DH, von Arnold S, Zhivotovsky B and Bozhkov PV: **Metacaspase-dependent programmed cell death is essential for plant embryogenesis.** *Curr Biol* 2004, **14:**R339–R340.

S7
Maintenance of the plant genome under natural light

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BMC Plant Biology 2005, **5(Suppl 1):**S7

Solar UV interacts with and alters a number of cellular components. Among these, DNA is perhaps the most critical and irreplaceable target. The majority of UV-induced damage takes the form of pyrimidine dimers. These lesions act both as blocks to the progression of both replicative DNA polymerases and the RNA polymerase holoenzyme. Expression of "dimer bypass" DNA polymerases may also lead to permanent changes (mutations) in DNA sequence, by inserting potentially incorrect bases opposite the lesion. Thus the excision or reversal of UV-induced damage is important both in the soma, as it is required for the maintenance of gene expression, and in the germline, where error-free repair pathways maintain genomic integrity. The basic mechanisms of photoreactivation and excision repair of UV-induced dimers are well understood in plants, although we would like to learn more about the tissue-specificity and environmental regulation of these important UV-protective mechanisms. Field experiments suggest that repair of UV-induced dimers is not essential to the survival of Arabidopsis plants, nor is the expression of the sinapic acid esters that act as natural sunscreens. However, plants that are defective in both repair and sunscreen production die within hours of exposure to natural light.

Progression through the cell cycle in the presence of unrepaired DNA damage products leads to a progressive deterioration of the genome. During S phase, persisting lesions are either miscopied or produce daughter strand gaps opposite dimers, which are difficult to repair in an error-free manner. For this reason, cells respond to persisting DNA damage by arresting the cell cycle in order to provide time prior to the initiation of S phase (G1/S arrest), the continuation of S phase (intra-S arrest) or progression into M (G2/M arrest). These DNA damage-induced cell cycle "checkpoints" are now being characterized in plants. Given the homologies between plant and mammalian damage checkpoint genes, it is likely that many aspects of cell cycle regulation by UV-induced damage are shared between plants and animals.

One UV-induced mammalian response to persisting DNA damage is the induction of programmed cell death. This actively induced apoptosis and necrosis of damaged cells leads to the inflammation observed in sunburn. The induction of cell death has two beneficial effects: the resulting bursts of radicals are thought to further stimulate the repair response of neighboring cells, and the suicide of damaged cells precludes their possible progression into cancer. Plant cells can also be killed by very high doses of UV light, in what might be a programmed response (as the genomic "laddering" characteristic of programmed cell death occurs), but it is not clear that such a response occurs under natural light. Given plants natural resistance to the lethal effects of cancer, it is possible that plants differ from mammals in this aspect of UV-response, and lack a sensitive apoptotic response to DNA damage.

S8
The FtsH protease is required for the repair of Photosystem II in the cyanbacterium *Synechocystis* 6803 damaged UV-B radiation

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BMC Plant Biology 2005, **5(Suppl 1):**S8

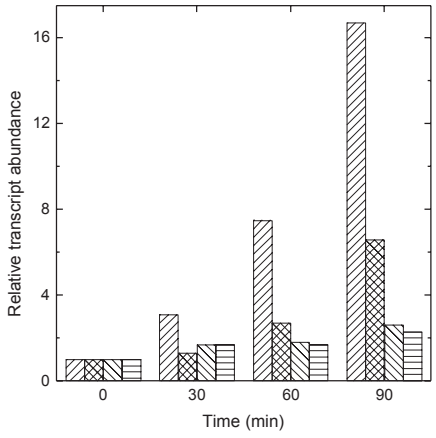
Background: UV-B radiation inhibits the electron transport of the PSII complex by damaging the Mn cluster of water oxidation and leads to the degradation of the D1 subunit of the

reaction center complex [1, 2]. In intact cells the inhibited PSII activity can be restored via *de novo* synthesis of the D1 subunit [3]. The FtsH protease has been shown to play an important role in the turnover of the D1 protein when induced by visible light both in higher plants and in cyanobacteria [4, 5]. However, the role of FtsH in the damage and repair of PSII under UV-B irradiation has not been clarified. Here we studied this question by using a mutant strain of *Synechocystis* 6803 cyanobacterium, which lacks the *slr0228* gene that encodes one of the FtsH homologues.

Materials and methods: *Synechocystis* sp. PCC 6803 cells were propagated in BG-11 growth medium in a rotary shaker at 30°C under a 5% CO_2-enriched atmosphere. The MFtsH mutant was constructed by interrupting the *slr0228* gene with a chloramphenicol resistance cassette as described in Ref. [5]. UV-B irradiation was performed in open glass containers in which the cell suspension of 6.5 Mg Chl ml^{-1} formed a layer of 10 mm height. A Vilbert-Lourmat VL-215M lamp was used as UV-B light source, with maximal emission at 312 nm, in combination with an 0.1 mm cellulose acetate filter (Clarfoil, Courtaluds Chemicals, UK) in order to screen out any UV-C contribution. The UV-B intensity was 4.8 Wm^{-2} ($\approx$ 13 $MEm^{-2}s^{-1}$) at the surface of the cell suspension. UV-induced changes in the transcript level of the FtsH homologue genes were detected by quantitative RT PCR. Light-saturated steady-state rate of oxygen evolution was measured using a Hansatech DW2 O_2 electrode at a light intensity of 1000 $MEm^{-2}s^{-1}$ in the presence of 0.5 mM 2,5-dimethyl-*p*-benzoquinone as electron acceptor.

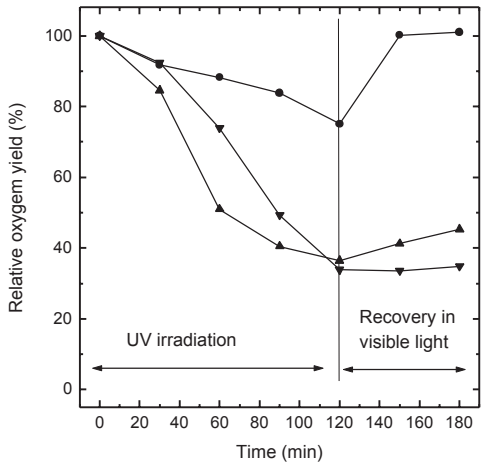
Results: The cyanobacterium *Synechocystis* 6803 has four homologues of the membrane bound ATP-dependent FtsH protease encoded by the *slr0228*, *slr1390*, *slr1604* and *sll1463* genes [6]. We have checked the effect of UV-B irradiation on the relative transcript level of these genes by quantitative RT PCR. As shown by the data in Figure 1 all four genes were induced more than 2-fold after 90 min UV-B exposure with the most significant effect seen in the *slr0228* and *slr1604* transcripts.

Figure 1 (abstract S8)



UV-B induced expression of the FtsH homologue genes. The transcript levels were detected by quantitative RT PCR for the *slr0228* (right hatch), *slr1604* (crossed hatch), *slr1390* (left hatch), *sll1436* (horizontal hatch) genes

Figure 2 (abstract S8)



UV-B induced loss, and recovery of oxygen evolution. The time course of oxygen evolution was followed in the WT (circles) and MFtsH cells (up triangles) during exposure to UV-B light and subsequent recovery in low intensity (40 $MEm^{-2}s^{-1}$) visible light. The time course of oxygen evolution is also shown in the presence of lincomycin for the WT cells (down triangles)

In search for the role of the FtsH protease we studied the UV-B induced damage in the oxygen evolving activity of PSII in the MFtsH mutant from which the *slr0228* gene had deleted. When compared to the WT, the MFtsH strain showed accelerated loss of oxygen evolution under UV-B exposure and almost complete lack of recovery under visible light (Fig. 2.). The loss of oxygen evolution in the MFtsH strain has practically the same kinetics as seen in the WT in the presence of the protein synthesis inhibitor lincomycin (Fig. 2.).

Conclusion: Our data demonstrate that the FtsH protease is required for the efficient repair of UV-damaged PSII centers in *Synechocystis* 6803 cells. Considering that restoration of PSII activity requires *de novo* synthesis of the D1 reaction center protein [3], we conclude that in the MFtsH strain the degradation and synthesis of the D1 protein is blocked.

Acknowledgements

This work has been supported in by a grant from the Hungarian Granting Agency OTKA (T034321).

References

1. Vass I, Sass L, Spetea C, Bakou A, Ghanotakis D and Petrouleas V: **UV-B induced inhibition of photosystem II electron transport studied by EPR and chlorophyll fluorescence. Impairment of donor and acceptor side components.** *Biochemistry* 1996, **35:**8964–8973.
2. Friso G, Spetea C, Giacometti GM, Vass I and Barbato R: **Degradation of photosystem II reaction center D1-protein induced by UVB irradiation in isolated thylakoids. Identification and characterization of C- and N-terminal breakdown products.** *Biochim Biophys Acta* 1994, **1184:**78–84.
3. Sass L, Spetea C, Motй Z, Nagy F and Vass I: **Repair of UV-B induced damage of Photosystem II via *de novo* synthesis of the D1 and D2 reaction centre subunits**

in *Synechocystis* sp. PCC 6803. *Photosynth Res* 1997, **54:**55–62.
4. Bailey S, Thompson E, Nixon PJ, Horton P, Mullineaux CW and Robinson C: **A critical role for the Var2 FtsH homologue of *Arabidopsis thaliana* in the photosystem II repair cycle *in vivo*.** *J Biol Chem* 2002, **277:**2006–2011.
5. Silva P, Thompson E, Bailey S, Kruse O, Mullineaux CW and Robinson C: **FtsH Is involved in the early stages of repair of photosystem II in *Synechocystis* sp PCC 6803.** *Plant Cell* 2003, **15:**2152–2164.
6. Sokolenko A, Pojidaeva E, Zinchenko V, Panichkin V, Glaser VM and Herrmann RG: **The gene complement for proteolysis in the cyanobacterium *Synechocystis* sp. PCC 6803 and *Arabidopsis thaliana* chloroplasts.** *Curr Genet* 2002, **41:**291–310.

S9

Use of RAPD assay for the detection of mutation changes in plant DNA induced by UV-B and γ-rays

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BMC Plant Biology 2005, **5(Suppl 1):**S9

Background: RAPD (random amplified polymorphic DNA) analysis is wide used for the genetic mapping, taxonomic and phylogenetic studies of many organisms. It can be also applicable for the detection of DNA alterations after influence of many genotoxical agents as well [1]. UV-radiation can produce several major types of DNA lesions such as cyclobutane-type pirimidine dimers and the 6-4 photoproducts [2]. Another important types of DNA damages such as protein cross-links, DNA strand breaks, deletion or insertion of base pairs can also be induced by UV-irradiation. Whereas, irradiation by γ-rays leads to the increasing level of DNA break formation. These different types of DNA damages must be detected by changes in RAPD profiles. The aim of present study was to investigate the possibility mutation changes in plant DNA after the influence of UV-B and γ-ray with using RAPD method. The 10-mer primers with single nucleotide substitutions were used in order to estimate these structural alterations in DNA following after genotoxical agents' treatment.

Materials and methods: Approximately 0.20 g of plant tissue from alfalfa seedlings, which was irradiated by high doses of UV-B and γ-ray (LD_{50}) was frozen in liquid nitrogen, ground with mortar and pestle and incubated in 1.5 ml of extraction buffer (100 mM Tris-HCl pH 8, 100 mM NaCl, 50 mM EDTA pH 8, 2% SDS and 0.1 mg/ml proteinase K) during 1.5 h at 37°C. DNA was extracted twice with chloroform:isoamyl alcohol (24:1) mixture. DNA was precipitated from the aqueous phase with 2 vol. of cold isopropanol at –20°C during 48 h. DNA pellet was harvested by centrifugation, washed several times in 70°ethanol, air-dried and dissolved in deionized water. RNAse treatment was performed accordingly to [3]. DNA was analyzed using agarose gel electrophoresis.

RAPD amplification involved initial denaturation of DNA template (20 ng per 25 μl reaction mix) at 94°C for 3 min, followed by 33 cycles of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C in thermocycler "Tertsik" (Russia). Amplification was finished with incubation at 72°C for 7 min. The sequences of 10-mer primers (MedBioservice) with single base substitutions are given in Table 1.

Table 1 (abstract S9) Sequences of the 10-mer primers (5'-3') used in experiments

Primer	Sequence
OPB 8	GTCCACACGG
OPB 8-1	G**A**CCACACGG
OPB 8-2	GT**G**CACACGG
OPB 8-3	GTC**G**ACACGG
OPB-8-4	GTCC**T**CACGG

PCR amplification products were analyzed in 2.5% (w/v) agarose gel in 0.5xTBE buffer. PCR products (25 μl per sample) were mixed with 3–5 μl Gel Loading Dye Solution (Fermentas, Lithvuania) and loaded onto the agarose gel, containing ethidium bromide (0.5 mkg/ml). Gene Ruler 100 bp DNA ladder was used for each agarose gel. Electrophoresis was carried out at 80 V for 3.5 h, then the results were visualized under UV light and documented using Canon digital camera.

Results: It was shown, that single nucleotide substitutions in 10-mer primers indeed can be reflected on the amplicons' profiles. The main changes observed in the RAPD profiles have been resulted both in an appearance (see Figure 1) or disappearance of different bands (see Figure 2) with variation of their intensity as well. These effects might be connected with structural rearrangements in DNA caused by different types of DNA damages.

Conclusion: Thus, RFLP method is applicable for the detection of changes in the DNA structure after different genotoxical treatments. The variation in band intensity and disappearance of some bands may correlate with level of photoproducts in DNA template after genotoxical treatment, which can reduce the number of binding sites for *Taq* polymerase. Appearance of new bands can be explained as the result of different DNA structural changes (breaks, transpositions, deletions etc). We can estimate the existence of mutation and structural alterations in plant DNA after impact of different stressful factors on the bases of DNA patterns obtained after RAPD with the set of primers. Obviously, sensitivity of the RAPD assay depends of the mutations level and it needs further investigations.

References

1. Atienzar F: **Evaluation of the RAPD assay for detection of DNA damage and mutations.** *Mut Res* 2002, **521:**151–163.
2. Hollosy F: **Effects of ultraviolet radiation on plant cells.** *Micron* 2002, **33:**179–197.
3. Draper J and Scott R: **Plant genetic transformation and gene expression.**Moscow: Mir Publ (in Russ); 1991.

S10

Nitric oxide functions in the plant hypersensitive disease resistance response

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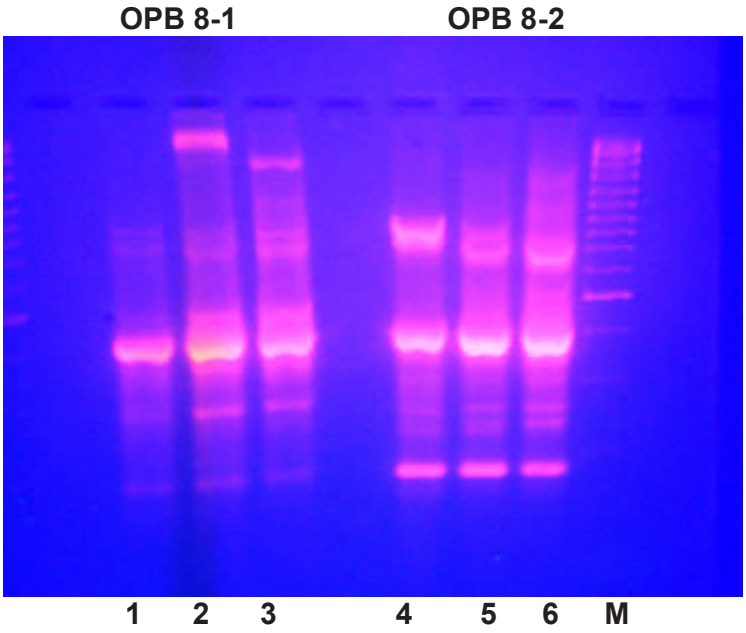
BMC Plant Biology 2005, **5(Suppl 1):**S10

Nitric oxide (NO) is a highly reactive molecule that rapidly diffuses and permeates cell membranes. In animals, NO is implicated in a number of diverse physiological processes such

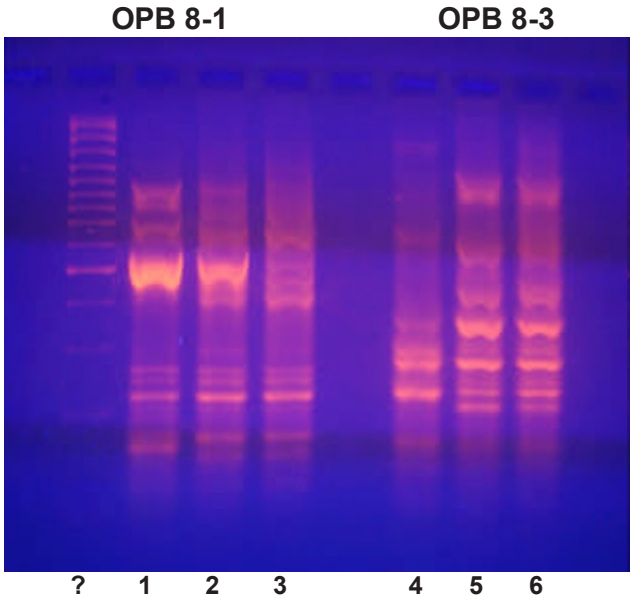
Figure 1 (abstract S9)



Effect of single nucleotide substitutions in 10-mer primers (OPB 8-1, OPB 8-2) on RAPD profile (**M** — Gene Ruler 100 bp DNA Ladder): **1, 4** — unirradiated control; **2, 5** — γ-irradiation; **3, 6** — UV-B

as neurotransmission, vascular smooth muscle relaxation, and platelet inhibition. It may have beneficial effects, for example as a messenger in immune responses, but it is also potentially toxic

Figure 2 (abstract S9)

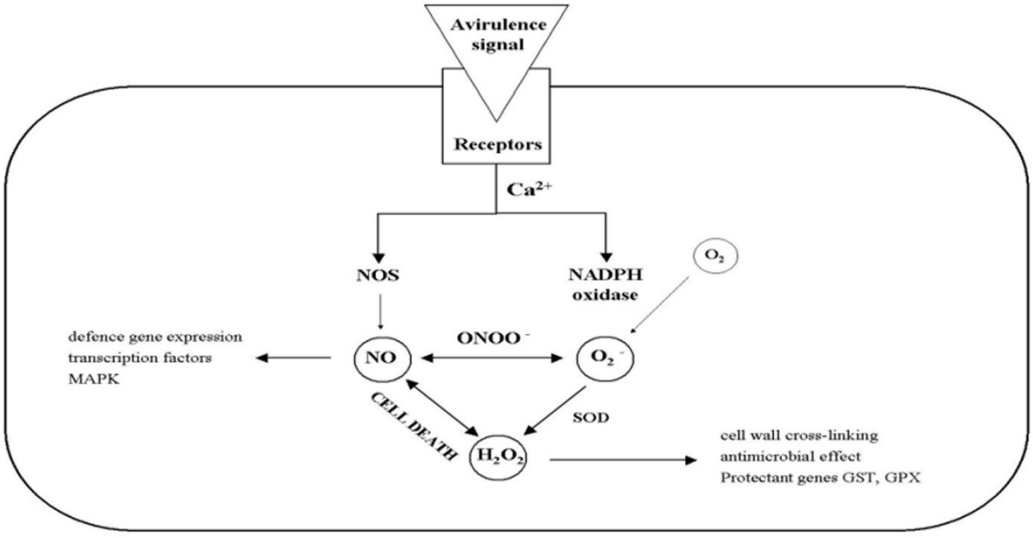


Effect of single nucleotide substitutions in 10-mer primers (OPB 8-1, OPB 8-3) on RAPD profile (**M** — Gene Ruler 100 bp DNA Ladder): **1, 4** — unirradiated control; **2, 5** — γ-irradiation; **3, 6** — UV-B

when the antioxidant system is weak and an excess of reactive oxygen species (ROS) accumulates. During the last few years NO as been detected also in several plant species, and the increasing number of reports on its function in plants have implicated NO as an important effector of growth, development, and defence [1]. The innate immune system of organisms as diverse as vertebrates, invertebrates, and plants shows several characteristics similar with respect to involvement of NO. In the mammalian immune system, NO cooperates with ROS to induce apoptosis of tumor cells and macrophage killing of bacteria. In plants a similar mechanism have evolved to prevent tissue invasion by pathogens. Attempted infection by an avirulent pathogen elicits a battery of defences often accompanied by collapse of challenged host cells thus delimiting the infected zone and avoiding the multiplication and spread of the pathogen. The rapid accumulation of ROS and NO through the activation of enzyme systems similar to neutrophil NADPH oxidase and nitric oxide synthase is one of the earliest events in this hypersensitive reaction (HR). Several lines of evidence suggest that death of host cells during the HR results from the activation of a cell death program, encoded by the plant genome and activated by a fine modulation of O_2^-, H_2O_2 and NO levels [2]. Both NO and ROS are necessary to trigger host cell death (see Figure 1); they are also components of a highly amplified and integrated defence system that involves activation of ion fluxes, changes in protein phosphorylation patterns, extracellular pH, membrane potential, oxidative cross-linking of plant cell wall proteins, and perturbations in the level of cytosolic Ca^{2+} that triggers the local expression of resistance genes [3].

NO also functions independently of ROS in the induction of various defence genes including pathogenesis-related proteins and enzymes of phenylpropanoid metabolism involved in the

Figure 1 (abstract S10)



NO and H_2O_2 mediated plant cell death. NO, nitric oxide; NOS, nitric oxide synthase; $ONOO^-$, peroxynitrite; H_2O_2, hydrogen peroxide; GPX, glutathione peroxidase; GST, glutathione S-transferase; SOD, superoxide dismutase; MAPK, mitogen-activated protein kinase

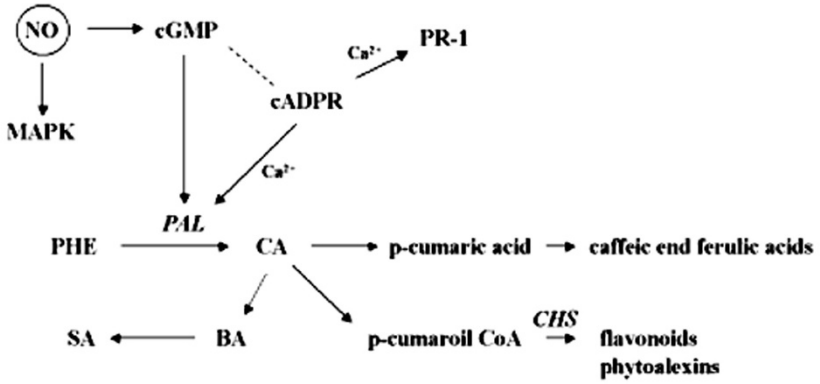
production of lignin, antibiotics and the secondary signal salicylic acid (see Figure 2). The mobile nature of NO and its chemical reactivity with various cellular targets means that downstream effects of NO may be directly induced by interaction with various cellular components, like ion channels or proteins that modulates gene expression, or indirectly following interaction with signalling proteins such as protein kinases. NO signalling functions depend on its reactivity and ROS are key modulators of NO in triggering cell death, although through mechanisms different from those commonly observed in animals [4].

In summary, although several hypotheses still await experimental demonstration, it is now clear that NO is an important component of plant defence systems. Much evidence supports the view that NO plays a key role in disease resistance responses. Moreover, the recent identification of a plant NOS will lead to the characterization and manipulation of mechanisms modulating NO signalling. Thus, the understanding of NO signalling functions at the biochemical, cellular and molecular levels will soon make it possible to discern several important physiological and pathological processes in plants, as has already been demonstrated in mammals.

References

1. Delledonne M, Xia Y, Dixon RA and Lamb C: **Nitric oxide functions as a signal in plant disease resistance.** *Nature* 1998, **394:**585–588.
2. Delledonne M, Murgia I, Ederle D, Sbicego PF, Biondani A, Polverari A and Lamb C: **Reactive oxygen intermediates modulate nitric oxide signaling in the plant**

Figure 2 (abstract S10)



Representation of NO signalling functions during the HR. CHS, chalcone synthase; CA, cinnamic acid; Ca^{2+}, calcium influx; cADPR, cyclic cADP ribose; cGMP, cyclic GMP; MAPK, mitogen-activated protein kinase; PAL, phenylalanine ammonia lyase; PHE, phenylalanine; PR, pathogenesis-related proteins; SA, salicylic acid; BA, benzoic acid. Dotted lines represent potential NO functions

hypersensitive disease-resistance response. *Plant Physiol Biochem* 2002, **40:**605–610.
3. Romero-Puertas MC and Delledonne M: **Nitric Oxide Signaling in Plant-Pathogen Interactions.** *IUBMB Life* 2003, **55:**579–583.
4. Delledonne M, Zeier J, Marocco A and Lamb C: **Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response.** *Proc Natl Acad Sci USA* 2001, **98:**13454–13459.

S11
Production of nitric oxide and reactions with plant hemoglobins under hypoxic stress

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BMC Plant Biology 2005, **5(Suppl 1):**S11

Nitric oxide (NO) is a reactive gas involved in many biological processes of animals, plants and microbes. The objectives of this study were: to examine whether NO is produced in transgenic maize suspension cell cultures and transgenic alfalfa roots exposed to hypoxic growth conditions, to determine whether there is a relationship between a class 1 hemoglobin and the amount of NO detected under hypoxic conditions, to estimate the route of formation and breakdown of NO in the tissue and to determine whether there is a relationship between NO production and aerenchyma formation. Maize suspension cell cultures and alfalfa roots, transformed to express the sense or antisense strands of barley hemoglobin were used to overexpress or underexpress class 1 hemoglobin. Up to 500 nmol NO/g fresh weight were detected in maize cells exposed to low oxygen tensions for 24 h. The steady state levels of NO in the different cell lines under hypoxic conditions had an inverse relationship to the level of hemoglobin in the cells. There was no detectable NO produced under aerobic growth conditions. Moreover EPR spectra showed evidence of nitrosylated heme complexes in denatured samples of hypoxically-exposed maize cells from both a wild type cell culture and a culture transformed to overexpress hemoglobin. No EPR signal characteristic of nitrosylated heme complexes was evident under aerobic conditions or in treated maize cells transformed to reduce hemoglobin expression. Spectroscopic data demonstrated that recombinant maize hemoglobin reacted with NO to form methemoglobin and NO_3^-. Nitrate was shown to be a precursor of NO in hypoxic maize suspension cell cultures by using $^{15}NO_3^-$ and EPR spectroscopy, suggesting that NO is formed via nitrate reductase during hypoxia. There was an inverse relationship between the expression of hemoglobin and the formation of aerenchyma under hypoxic conditions. The Hb^- line displayed strong evidence of aerenchyma formation under hypoxia, whereas an Hb^+ line showed only slight evidence of cell breakdown characteristic of aerenchyma formation. The levels of NO expressed distal to the root tip were, at least, five fold greater than those found in the tip. The results demonstrate that NO is produced in plant tissues grown under low oxygen tensions and suggest that class 1 hemoglobins have a significant function in regulating NO levels.

S12
Nitric oxide, cell death and increased taxol recovery

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BMC Plant Biology 2005, **5(Suppl 1):**S12

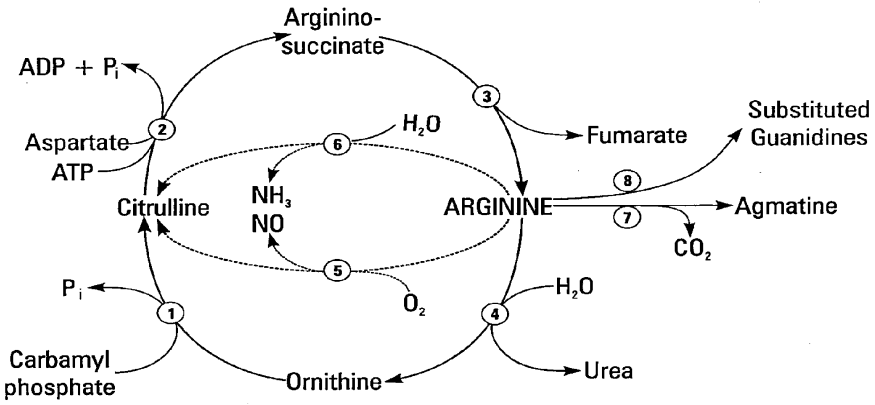
Background: With NASA support this research aimed at evaluating early opportunities in Microgravity Sciences to commercialize space and to develop the biotechnology facility for the International Space Station [1]. The main task was to evaluate the production of taxol (generic name: paclitaxel) with cell suspensions in bioreactors designed for the Space Shuttle. Unexpectedly, this work led to the early demonstration of L-arginine-dependent nitric oxide (NO) bursts in mechanically and gravitationally stressed plant cells, to NO-induced programmed cell death (apoptosis) (reviewed in [2]), and to a model describing how these factors contribute to increased taxol and taxane recovery from conifers.

Earlier, when intermediates of the Krebs-Henseleit or urea cycle (See Figure 1) were fed to conifers, several substituted guanidino compounds were derived from uniformly labeled ^{14}C-L-arginine, and less so from ^{14}C-L-citrulline [3, 4]. At that time, the substituted guanidines were considered mainly as respiratory inhibitors [5, 6]. Today, they are natural inhibitors of plant, animal and human nitric oxide synthases (NOSs). NOS substrates are L-arginine and oxygen. NOS products are L-citrulline and NO.

Since nitrate and nitrite reductases were also known sources of NO, we obtained an *Arabidopsis* nitrate reductase double mutant with the aim of finding out if cells could produce NO in the absence of nitrate, nitrite, and their reductases [7]. With this mutant we reaffirmed that the source of NO was putative NOS activity. The production of NO from L-arginine was blocked by D-arginine, and by the NOS inhibitor, N^G-monomethyl-L-arginine (L-NMMA) ensuring that NO was produced in the absence of any residual nitrate reductase activity. NOS-dependent NO production in cells was inhibited by other guanidino compounds but not by D-arginine. Subsequently, the discovery by others of two plant NOS genes provided evidence that plant genomes code for NOS. In our work with *Taxus* cell suspensions, the substituted guanidines offered protection against mechanically induced stress and cell-death or apoptosis.

Taxol is an effective anti-cancer agent that was first isolated from the bark of *Taxus brevifolia* [8]. Taxol binds to microtubules thereby offering a novel mechanism of blocking cell proliferation. It became the best-selling anticancer drug in history. By 2000, commercial sales of taxol were well over $1.5 billion. New models for taxol and taxane biosynthesis emerged [9] so that taxol biosynthesis could be followed at the subcellular level by immunocytochemistry, and by laser confocal and scanning electron microscopy [10, 11, 12, 13, 20]. The use of NO donors, NOS substrates, products, inhibitors (substituted guanidines), and NO traps provided new opportunities to control the citrulline-NO cycle (See Figure 1), apoptosis, and taxol production in unit gravity, simulated microgravity, and in hypergravity.

Figure 1 (abstract S12)



The reactions of the Krebs-Henseleit (urea) cycle, and their relations to NOS activity. Enzymes: **1.** ornithine carbamoyl transferase, **2.** argininosuccinate synthetase, **3.** argininosuccinate lyase, **4.** arginase, **5.** nitric oxide synthase, **6.** arginine deiminase, **7.** arginine decarboxylase, **8.** numerous enzymes contributing to the formation of substituted guanidino compounds. Reactions 1 to 4 comprise the urea cycle. Reactions 2, 3, 5, may account for NOS activity in plants (citrulline-NO cycle). Arginine deiminase was reported in chloroplasts but is mostly found in microorganisms. Reactions 7 and 8 comprise decarboxylation, oxidation, methylation, transamidination, phosphorylation, keeping the guanidino group intact or modifying it by methylation, phosphorylation, etc. They remove L-arginine as a substrate from the urea cycle, and from reactions 5 and 6. L-arginine represents an important branch point that links nitrate and ammonium nutrition to protein synthesis and turnover (not shown), to the urea cycle, to a postulated citrulline-NO cycle, and to the formation of guanidino compounds (substituted and non substituted). Through oxygen requirements, the stress-induced NOS activity links respiration to NO, ROS and RNS production, their signaling pathways and damaging reactions, e.g., the nitration of phenols and tyrosines residues in cell regulatory proteins, and to apoptosis.

Haploid egg cells from female trees of *T. brevifolia* were selected as the experimental material [12, 19]. These cells are easily screened without the effects of dominance, recessive, and epistatic interactions characteristic of diploid cells. Lethal genes are directly expressed, and removed by apoptosis making cell populations genetically more uniform. Diploid cell suspensions were also established from *T. cuspidata* [13] needles on 3-year-old stock obtained in 1995 from Zelenka Nursery (Grand Haven MI), and from seeds of *T. chinensis*. This provided better comparisons with all other published work with using diploid cells.

Cell suspensions were maintained in darkness at 25 ± 2°C on semisolid media, in 125 ml Erlenmeyer flasks (60 rpm), 1 L nippled flasks on a clinostat (1 rpm simulating $2 \times 10^{-4} \times g$, but with significant convective mixing of the gaseous and liquid environment), 100 ml high-aspect rotating vessels (HARV 12.5 cm dia.), and rotating cylindrical culture vessels (RCCV 7.5 cm dia.) both at ca.$10^{-2} \times g$ (Synthecon, Houston TX). The HARV and RCCV were used by NASA in early space shuttle experiments, and in a mini-payload integration center, designed as an in-flight laboratory.

In all cell assays, apoptotic cells were distinguished morphologically and by the TUNEL reaction [14, 15]. Free taxol, taxanes, and baccatin III in cells and the culture medium, or bound taxol and taxanes, released after xylanase activity, were determined with competitive inhibition enzyme-linked immunoassays kits from Hawaii Biotechnology. Cells were examined by laser confocal microscopy (Zeiss LSM 410 Invert Scan Microscope) using single or double-labeling immunocytochemical fluorescence (FITC, Cy3) and colloidal gold to reaffirm the subcellular locations of taxol, the taxane ring (baccatin III), the C-13 side chain of taxol, and taxanes in general. For samples larger than 10 g fresh biomass, these compounds were determined by HPLC using authentic standards and taxil columns (MetaChem Technologies Inc.) [12].

Results: With *T. cuspidata* suspension in RCCV bioreactors ($10^{-2} \times g$), and in 1 L nippled flasks (3 wks in darkness at 24°C), free taxol comprised 42 and 21 percent of the total taxanes (1.1 and 2.4 mg taxol/kg air-dry biomass weight), respectively [13]. Centrifugation of cells at 3 and 24 × g greatly increased taxane content but reduced taxol recovery. *T. cuspidata* and *T. brevifolia* responded similarly to mechanical stresses, simulated microgravity, and hypergravity by producing NO bursts, apoptosis, and by the overproduction of taxol and taxanes.

T. brevifolia heat-killed cells did not produce NO. Generally 19% of the live cells (unit gravity in shaker flasks for 3 h) were always positive for NO as assayed with DAF-2DA. With the addition of 10^{-4} mM SNP, a NO donor, or after centrifugation at 150 × g for 3 h, nearly all cells produced NO. However, if 0.5 mM L-NMMA (NOS inhibitor) was added, only 26% of the cells produced NO. When 0.5 mM D-NMMA, a non inhibitor of NOS, was added, 92% of the cells produced NO [14].

The greater the NO produced and released by cells, the more cells became apoptotic. Additions of SNP (10^{-6} to 10^{-2} mM) increased apoptosis from 18 to 75%. Centrifugation at 150 g yielded 35% apoptosis. This was reduced to less than 10% by the addition of the NOS inhibitor, L-NMMA. Substituting D-NMMA for L-NMMA resulted in apoptosis (32% cells), but this was not significantly different from the cells exposed to 150 g [14]. The NOS requirement for oxygen connected NO production to the stress-induced and damaging increases in free radicals, *viz*., reactive oxygen species and reactive nitrogen species [2]. The lesson learned offered guanidino compounds as countermeasures to NO-mediated stress and apoptosis.

At unit gravity and after 10 d, egg cells exposed to SNP (10^{-4} mM) produced 18 mg taxol/Kg fresh weight. This taxol yield was now 64% higher than the controls without SNP. The number of cells producing NO with SNP was 86.7% higher than the controls without SNP. By contrast, the addition of L-NMMA (0.5 mM) decreased NO production by 87.3%. Taxol yield was reduced by 75% (4.1 mg/Kg fresh weight) (unpublished results).

In a separate study at unit gravity with cells supported on filter paper, the effects of SNP (10^{-4} mM), sodium tungstate, (a nitrate reductase inhibitor at 1 mM), and L-NMMA (0.5 mM) were used to evaluate taxol yields after 5 d. SNP added alone, or tungstate with SNP together, yielded taxol at 8.0 and 8.1 mg/Kg fresh weight, respectively, i.e., the differences were not significantly different. This reaffirmed that nitrate reductase activity was not a contributor to NO-dependent taxol production. L-NMMA alone, or together with added tungstate, reduced taxol recovery to 2.1 and 2.0 mg/kg fresh weight, respectively. Here again, NOS was responsible for the NO that increased taxol production without a significant contribution from nitrate reductase. Removing and starving the control egg cells only slightly increased taxol recovery.

The tetracyclic diterpenoid ring of taxol is synthesized by cyclization of geranylgeranyl diphosphate to give taxa-4(5),11 (12(-diene) in plastids and in light and darkness [9, 11]. This basic ring structure gives rise to over 300 different taxanes. One of these, baccatin III, can provide the ring structure for the biosynthesis of taxol. Although the mechanisms are not yet clear, taxol formation also requires the addition of a C-13 side-chain originating from phenylalanine. Additional and finishing structural changes for taxol involve reaction in the endoplasmic reticulum and Golgi stacks.

Using specific antibodies, the biosynthesis of the taxol ring in plastids, and the cytoplasmic assembly of bound taxol and related taxanes were visualized by laser confocal and scanning electron microscopy. Gold labeling was especially useful to identify taxol and taxanes in transport vesicles, the plasmalemma, cell walls, and in the culture medium. Colloidal 40 nm-gold-immunolabeled antibodies to taxanes, taxol, and baccatin III were visualized with 7 to 21 nM sensitivity, with and without gold backscatter (laser confocal and scanning electron microscopy).

In egg cells, baccatin III (taxane ring) was detected in gravisensing amyloplasts and vesicles associated with plastid membranes. Baccatin III, taxol, and other taxanes were transported through the cytoplasm by these vesicles, which then released their contents to the plasma membrane. Plastids, that moved and docked at the plasma membrane, also released bound taxol and taxanes into the plasma membrane. The bound taxol and taxanes at the cell-surface were deposited in newly formed cell walls or released into the culture medium. The drug-productive cells and materials in the culture medium containing bound taxol, baccatin III, and taxanes were collected with antibody-labeled paramagnetic beads [16]. Immunogold-labeled taxol, taxanes and baccatin III were detected in transport vesicles, plasma membrane fragments, and on materials recovered from the culture medium.

Bound taxol and taxanes were recovered from xylanase (commercial and purified) hydrolysates of cell biomass, debris in the culture medium, and from the wood of trees. Taxol and other taxanes were characterized and identified by HPLC using authentic standards. Results indicated that recovered taxol was bound to xyloglucan oligosaccharides. Probes of cells with an antibody to xyloglucan endotransglycosylases implied that 'touch' genes normally expressed under mechanical forces [18], could have provided additional sites for the attachment of taxol and other taxanes.

In hypergravity (3 to 150 × g), the taxol and taxanes released from cells by syneresis, were recovered on hydrophobic PVDF (polyvinylidene fluoride) filters. Cyclodextrins added to the culture medium enhanced biomass yield and altered the solubility of taxanes to improve the recovery of taxol and taxanes. The addition of cyclodextrins to the culture medium improved cell biomass and taxol recovery [17]. Further details are available in US Patents [16, 17, 18, 20].

Acknowledgements

This work was impossible without the support from NASA's Microgravity Grant No. 9-825. Special thanks go to Mr. Frank Ventimiglia for determining and locating taxol in the cell suspensions. Dr. Cristina Pedroso and Helena Garcκs carried out the NO, TUNEL and NOS- related studies. Drs. H-C Dong and C. Pedroso contributed to the taxol assays using the protocols worked out by F. Ventimiglia. A post-doctoral fellowship from China for Dr. Dong (China) aided in studies with NO and taxol. Dr. Dorward at the National Institutes of Health, Rocky Mountain Laboratory, prepared the scanning electron micrographs.

References

1. Durzan DJ: **Metabolic engineering plant cells in a space environment.** *Biotechnol Genet Eng Rev* 2000, **17:**349–383.
2. Durzan DJ and Pedroso MC: **Nitric oxide and reactive nitrogen oxide species in plants.** *Biotechnol Genet Eng Rev* 2002, **19:**293–337.
3. Durzan DJ: **Nitrogen metabolism of *Picea glauca*. I. Seasonal changes of free amino acids in buds, shoot apices and leaves, and the metabolism of uniformly labeled ^{14}C-L-arginine by buds during the onset of dormancy.** *Can J Bot* 1968, **46:**909–919.
4. Durzan DJ: **Nitrogen metabolism of *Picea glauca*. IV. Metabolism of uniformly labeled ^{14}C-L-arginine, [carbamyl-^{14}C]-L-citrulline, and [1,2,3,4-^{14}C]-γ guanidinobutyric acid during diurnal changes in the soluble and protein nitrogen associated with the onset of expansion of spruce buds.** *Can J Biochem* 1969, **47:**771–783.
5. Durzan DJ and Steward FC: **Nitrogen metabolism.** *Plant Physiology, An Advanced Treatise* Academic Press, Inc., New York: Steward FC 1983, **8:**55–265, ***Chapt.*** 2.
6. Bidwell RGS and Durzan DJ: **Some recent aspects of nitrogen metabolism.** *Historical and Recent Aspects of Plant Physiology: A Symposium Honoring FC Steward* Cornell Univ Press, College Agriculture and Life Science; 1975, 162–227.
7. Garcκs H, Durzan DJ and Pedroso MC: **Mechanical stress elicits nitric oxide formation and DNA fragmentation in *Arabidopsis thaliana*.** *Annals Bot* 2001, **87:**567–574.
8. Goodman J and Walsh V: ***The story of taxol. Nature and politics in the pursuit of an anti-cancer Drug.***Cambridge Univ Press, Cambridge; 2001, 282.
9. Jennewein S and Croteau R: **Taxol: biosynthesis, molecular genetics, and biotechnological applications.** *Appl Microbiol Biotech* 2001, **57:**13–19.
10. Grothaus P, Bignami GS, O'Malley S, Harada KE, Byrnes JB, Waller DF and Raybould TJ: **Taxane-specific monoclonal antibodies: Measurement of taxol, baccatin.** *Nat Prod* 1995, **58:**1003–1004.

11. Durzan DJ: **Gravisensing, apoptosis, and drug recovery in *Taxus* cell suspensions.** *Gravit Space Biol Bull* 1999, **12:**47–55.
12. Durzan DJ and Ventimiglia F: **Free taxanes and the release of bound compounds having taxane antibody reactivity by xylanase in female, haploid-derived cell suspension cultures of *Taxus brevifolia*.** *In Vitro Plant Cell Devl Biol* 1994, **30P:**219–227.
13. Durzan DJ, Ventimiglia F and Havel L: **Taxane recovery from cells of *Taxus* in micro- and hypergravity.** *Acta Astronautica* 1998, **42:**455–463.
14. Pedroso MC, Magalhaes JR and Durzan DJ: **Nitric oxide induces cell death in *Taxus* cells.** *Plant Sci* 2000, **157:**173–181.
15. Havel L and Durzan DJ: **Apoptosis in plants.** *Bot Acta* 1996, **109:**268–277.
16. Durzan DJ and Ventimiglia F: **Recovery of taxanes from plant material.** *US Patent* 1999, ***No.5,981,777.*** November 9. (Use of paramagnetic beads).
17. Durzan DJ and Ventimiglia F: **Cyclodextrins in plant nutrient formulations.** *US Patent* 2000, ***No.6,087,176.*** July 11.
18. Durzan DJ and Ventimiglia F: **Taxane production in haploid-derived cell cultures.** *US Patent* 1996, ***No.5,5547,866.*** November 9.
19. Antosiewicz DM, Purugganan MM, Polisensky DH and Braam J: **Cellular localization of *Arabidopsis* xyloglucan endotransglycosylase-related proteins during development and after wind stimulation.** *Plant Physiol* 1997, **115:**1319–1328.
20. Durzan DJ and Ventimiglia F: **Recovery of taxanes from conifers.** *US Patent* 1999 ***No.5,955,621.*** September 21. (screening taxanes from *Coniferales*).

S13
Diploid parthenogenesis and 'early embryo' development in Norway spruce cell suspensions

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BMC Plant Biology 2005, **5(Suppl 1):**S13

In diploid parthenogenesis, 'proembryos' developed from binucleate egg-equivalents containing an egg nucleus and an apoptotic ventral canal nucleus. As the axial tier of 'early embryos' formed, the cell regulatory proteins in proembryonal cells were ubiquitinated and turned over as suspensors differentiated. Axial tier formation was blocked at high levels of chlorsulfuron, an inhibitor of acetolactate synthase. This enzyme is required for the biosynthesis of branched-chain amino acids, which are especially abundant in ubiquitin. The block of acetolactate synthase led to the accumulation of free ∀-amino-n-butyrate. The overall behavior of branched-chain amino acids revealed rigid and linear relations over all chlorsulfuron levels. The proliferating cell nuclear antigen (PCNA), required for DNA synthesis, was detected in rapidly cycling proembryonal cells. PCNA appeared to serve as a factor maintaining the cell replication typical of rapidly growing early embryos. Less than 0.01% of nuclei reacted with epitopes to anti-p53 and anti-p21 that are commonly associated with cell cycle arrest and DNA damage. The cleavage sites of early embryos involved apoptosis and contributed to their multiplication. Chlorsulfuron contributed to aborted axial tier development due to disrupted patterns for the ubiquitination of cell regulatory proteins as revealed by changes in the soluble amino acids.

S14
New approach to radiation amplification factor

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BMC Plant Biology 2005, **5(Suppl 1):**S14

One important factor for the estimation of climate change is the Radiation Amplification Factor (RAF) defined as the percentage increase in UVbio that would result from 1% decrease in the column amount of atmospheric ozone [1, 2]. RAF can generally be used only to estimate effects of small ozone changes, e.g. of a few percent, because the relationship between ozone and UVbio becomes non-linear for larger ozone changes. In other words RAF is ratio of relative changes of UVbio and the ozone column. We can say also that RAF is differential slope of UVbio to the ozone column relationship in log-log scale.

It is necessary to note that the main peculiarity of this approach is its dimensionless properties. This enables us to compare values with various and different physical meanings. RAF values can give additional possibilities or information. A detailed and comparative analysis of dimensionless RAF values (from the point of denentionless nature) can give the following results.

1) A relatively strong influence and superlinear dependence is observed for: skin erythema, photocarcinogenesis, fish melanoma, generalized DNA damage, mutagenicity and fibroblast killing, cyclobutane pyrimidine dimer formation, occupational exposure limits, membrane-bound K+-stimulated ATPase inactivation, isoflavonoid formation in bean, inhibition of motility (*Euglena gracilis*), tropospheric photolysis O_3+hn-O(D')+O_2.

2) An approximately linear dependence was found for: SKH-1 corrected for human skin transformation, elastosis, damage to cornea, immune suppression, tropospheric photolysis HNO_3+hn-OH+HNO_2, photodegradation of nitrate ions, photodegradation of HCHO (Biscayne Bay), UVB (280–315 nm).

3) A weak dependence is for: fibroblast killing, substrate binding in Chinese hamster, DNA damage in Alfalfa, tropospheric photolysis H_2O_2+hn-OH+OH, HCHO+hn-H+CHO, yellowness induction in polycarbonate.

4) A very weak dependence was found for: melanoma in fish, photosynthetic electron transport, inhibition in photosynthesis (*Phaoedactylum sp.*), tropospheric photolysis O_3+hn-O(3P)+O_2, HCHO+hn-H_2+CO, photoproduction of H_2O_2 in fresh water.

Acknowledgements
This work was done in the frame of STCU project #1556.

References
1. Madronich S: **The radiation equation.** *Nature* 1995, **377:**682.
2. Madronich S, McKenzie RL, Caldwell MM and Bjom LO: **Changes in ultraviolet radiation reaching the earth's surface.** *Ambio* 1995, **24:**143–16.

S15
Signals from reactive oxygen species

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BMC Plant Biology 2005, **5(Suppl 1):**S15

Reactive oxygen species (ROS) can arise from normal metabolic activity such as organelle-based electron transport or be intermediates in signal transduction pathways activated by plant respiratory burst oxidase homologue (*Rboh*). UV-B exposure induces a pathogenesis-like response in leaves that can be abrogated by ROS scavengers [1]. A local signal is propagated by hydrogen peroxide and is sensitive to the application of catalase [2]. Similarly, local ROS production initiated by elicitors or pathogens can arise from stimulation of superoxide producing Rboh activity [3, 4]. In this case as well, propagation of a ROS signal to adjoining cells is sensitive to catalase. Antisense of Rboh homologues in tomato lead to reduced ROS production [5]. The tomato plants show compromised wound-dependent responses. In addition, the antisense plants have a highly branched phenotype and fasciated-like reproductive organs. Transcriptome analysis of these plants revealed ectopic expression of homeotic MADS box genes that are normally expressed only in the reproductive organs. In addition, various applications of hormones were found to regulate Rboh levels. Thus, regulated ROS bursts and the general effect of Rboh activity on the steady state cellular redox milieu control short term physiological reactions and plant development. Divergent stress including temperature, drought and UV-B exposure yield overlapping transcriptome response profiles whose origin can be traced to the use of reactive oxygen signaling intermediates. Cellular scavenging systems and local production of NO are likely to temper these signalling properties by interacting with ROS and thus help to contribute to the specificity of particular responses.

References
1. Green R and Fluhr R: **UV-B induced PR-1 accumulation is mediated by active oxygen species.** *The Plant Cell* 1995, **7:**203–212.
2. Allan AC and Fluhr R: **Two distinct sources of elicited reactive oxygen species in tobacco epidermal cells.** *The Plant Cell* 1997, **9:**1559–1572.
3. Allan AC, Lapidot M, Culver JN and Fluhr R: **An early TMV-induced oxidative burst in tobacco suggests an extracellular receptor for the virus coat protein.** *Plant Physiol* 2001, **126:**97–108.
4. Sagi M and Fluhr R: **Superoxide production by the gp91^{phox} NADPH oxidase plant homologue: modulation of activity by calcium and TMV.** *Plant Physiol* 2001, **126:**1281–1290.
5. Sagi M, Davydov O, Orazova S, Yesbergenova Z, Ophir R, Stratman JW and Fluhr R: ***Rboh* impinges on wound responsiveness and development in tomato.** *The Plant Cell* 2004, **16:**616–628.

S16
Organogenic nodule formation from hop internodes. Reactive oxygen species and expression of lipoxygenase and extracellular-regulated kinases

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BMC Plant Biology 2005, **5(Suppl 1):**S16

Organogenic nodules have been studied in several plant species as an additional morphogenic pathway for regeneration strategies, automated micropropagation, and genetic transformation for desirable characteristics. This process has been described in hop [1]. Lipoxygenases (LOX) have several functions in plants such as response to wounding stress and pathogen attack, and growth and development [2]. Western blotting analysis showed a *de novo* synthesis of LOXs isoenzymes in response to wounding and during development of nodules. Immunogold labelling detected LOX in peroxisomes of some nodular cells, probably related to programmed degradation of these organelles [3]. Nodule separation into "daughter nodules" was initiated by the formation of necrotic layers at the future region of nodule separation which accumulated reactive oxygen species (ROS) as revealed by NBT staining. Peroxisomes may have a function in plant cells as a source of signal molecules like nitric oxide and superoxide radicals [4]. Low levels of nitric oxide and ROS are involved in many physiological processes, including growth and development [4]. Preliminary studies showed that internodes cultured with 0.3 mM of N-acetylcysteine, an inhibitor of ROS, did not undergo nodule formation. LOX presence in peroxisomes may act as a source of ROS.

MAPK homologues belonging to the Extracellular-Regulated Kinases cascade were active either in the nucleus or cytoplasm depending on cellular type and developmental stage of nodule formation. ERK2 was activated upon wounding of internodes, apparently due to the formation of reactive oxygen species. ERK1 expression increased during nodule formation and plantlet regeneration [5]. During prenodule formation, ERKs were detected in the cytoplasm and nucleus of dividing cells, co-localizing with activated MAPKs. The presence of ERKs in the nucleus of prenodular cells may be related to the initiation of cell differentiation into nodules. Prenodules showed elevated levels of ROS. In vacuolated cells of nodules, ERK1/2 appeared in the cytoplasm and nucleus whereas in meristematic nodular cells they appeared only in the cytoplasm. These different cellular localization patterns may be related to proliferation and/or differentiation processes occurring during organogenic nodule formation.

References
1. Fortes AM and Pais MS: *Am J Bot* 2000, **87:**971–979.
2. Feussner I and Wasternack C: *Annu Rev Plant Biol* 2002, **53:**275–297.

3. Fortes AM: *J Histochem Cytochem* 2004, **52:**227–241.
4. del Río LA: *J Exp Bot* 2002, **53:**1255–1272.
5. Silva MF: *Eur J Cell Biol* 2004.

S17
UVB and UVA effects on both aquatic organisms enzymes and enzymatic reactions made by pure enzymes

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BMC Plant Biology 2005, **5(Suppl 1):**S17

In our research we found out that ultraviolet B irradiation increases the activity of the pure hydrolase (β-amylase, E.C.3.2.1.1) and Merck peroxidase (E.C.1.11.1.7) by free radicals generated from synthetic polymers walls of the experimental tubes. The activation was higher after UVB irradiation than after UVA. UVB and UVA increase the intensity of cell structure destruction by free radical-activated hydrolase determined on nude alga *Tetraselmis suecica*, cultivated bacteria *Escherichia coli* O_{157} (exponential phase), *Acinetobacter calcoaceticus* and total germs after the short time of exposure in the thermo-stated conditions.

The nude alga *T. suecica* was more resistant and stores starch and lipids. This alga was able to convert the energy of irradiation into chemical energy of synthetic products. Some individuals of *T. suecica* become encysted through creating of thick inhomogeneous sub-silique, under which a new silique appears. Other cells increase their glucide (intra-plastid starch granules) and lipid reserves of provisions (oleosoma appear in the central part of the cell and affect the tillakoid lamellar structure; plasto-globules appear as well). *T. suecica*, which lives in the surface region of the sea's waters, has 10 times higher growth resistance to natural solar irradiation. After 10 min. of exposure, it succeeds to convert energy of the absorbed radiation into the energy of the provision products stored as starch and lipid granules.

Cultivated bacteria on poor specific media developed very slowly after irradiation (transmittance 300–800 nm). If the bacteria were cultivated on reach media, which absorb UV (Martin medium) they are developed by n^3 rule, instead n^2 in the first stage, after irradiations of bacteria culture (transmittance 235–800 nm).

S18
Caspase-like activities and UV-induced programmed cell death in *Arabidopsis*

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BMC Plant Biology 2005, **5(Suppl 1):**S18

A very important goal is to determine which molecular components may be used in the execution of programmed cell death (PCD) in plants, which have been conserved during evolution and which are plant specific. Using *A. thaliana* we have shown that UV radiation can induce apoptotic-like changes at the cellular level and that an UV experimental system was relevant to the study of PCD in plants. UV induction of PCD requires light and a protease cleaving the caspase substrate Asp-Glu-Val-Asp (DEVDase activity) is induced within 30 minutes and peaks at one hour. This DEVDase appears related to animal caspases at the biochemical level, being insensitive to broad-range cysteine protease inhibitors. In addition, caspase1, caspase-3 inhibitors and the pancaspase inhibitor *p35* were able to suppress DNA fragmentation and cell death. These results suggest that a YVADase (Tyr-Val-Ala-Asp) activity and an inducible DEVDase activity are possibly mediating DNA fragmentation during plant PCD induced by UV overexposure. Progress is being made towards the biochemical characterisation of the proteases involved.

References

1. Danon A and Gallois P: **UV C radiation induces apoptotic like changes in *Arabidopsis thaliana*.** *FEBS Lett* 1998, **437:**131–136.
2. Danon A, Rotari V, Gordon A, Mailhac N and Gallois P: **UV-C overexposure induces a programmed cell death in *Arabidopsis*, which is mediated by caspase-like activities and can be suppressed by caspase inhibitors, p35 and defender against apoptotic death.** *J Biol Chem* 2004, **279:**779–87.

S19
Involvement of ethylene, oxidative stress and lipid-derived signals in cadmium-induced programmed cell death in tomato suspension cells

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BMC Plant Biology 2005, **5(Suppl 1):**S19

Background: Extensive research is ongoing looking for the characterisation of programmed cell death (PCD) in plants involving pathogen attack, chemical elicitation and abiotic inducers, but there are still limited reports on the role of heavy metals in PCD induction and little is known about cadmium-triggered signal transduction in plant systems. Contamination of biosphere with heavy metals has hazardous effect on agricultural crops and human health. In animal models, cadmium intoxication occurs through apoptosis appearing by apoptotic phenotype and an oxidative stress is involved in the mechanism of Cd action. The goal of this present work was to investigate if programmed cell death occurs in cadmium-treated tomato suspension cells; to identify some of the biochemical processes contributing to the signal transduction pathway(s) involved in cadmium toxicity; to investigate the role of oxidative stress (hydrogen peroxide), ethylene and lipid-derived signals and to look for similarities between cadmium- and camptothecin-induced cell death.

Materials and methods: The experiments were undertaken with tomato suspension cells, line Msk8. Specific inhibitors of different biochemical steps were administrated simultaneously with either $CdSO_4$ or topoisomerase-1 inhibitor camptothecin (CPT). Cell viability (FDA staining of the viable cells) was determined after 24 hours and the dynamics of H_2O_2 production was measured by chemiluminescence in a ferricyanide-catalised oxidation of luminol. Specific caspase peptide inhibitors, antioxidants, NADPH oxidase inhibitors, calcium channel

blockers, inhibitors of phospholipid cycle, protein kinase inhibitor and ethylene blockers were tested. Ethylene was applied during 24 h in concentrations up to 100 ppm in the head space. For details on methodology see [1, 2].

Results: The human caspase-1 inhibitor Ac-YVAD-CMK and the broad range caspase inhibitor Z-Asp-CH_2-DCB, abolished the cell death of Cd-treated and CPT-treated cells (See Table 1). This strongly suggests that the cell death pathway that is induced by cadmium employs caspase-like proteases and gives a reason to assume that in tomato suspension cells Cd-triggered cell death most probably resembles features of programmed cell death. The amount of hydrogen peroxide increased in response to Cd and CPT. Efficient inhibition of cell death occurred at the application of antioxidants and calcium channel blocker (See Table 1). The inhibition of NADPH oxidase by imidazole, quercetin and kaempferol significantly reduced the percentage of dead cells.

Treatments with ethylene further decreased both Cd- and CPT-reduced cell viability. Comparative experiments with Cd- or CPT-treated cells revealed an analogy in cell response to the ethylene inhibitor AVG (See Table 2). AVG greatly reduced the cell death that was enhanced in response to Cd or CPT. An increase of endogenous ethylene production (measured by laser photoacoustics) occurred in cadmium-treated cells. The data are a clear demonstration of ethylene involvement in Cd- and CPT-triggered cell death. Administration of IP_3 cycle inhibitors showed a strong inhibition to Cd-induced cell death.

Conclusion: Evidence is accumulating that caspase-like cysteine proteases showing functional similarity to animal caspases, participate in the programmed cell death in plants. In addition to discoveries that caspase-like proteases are involved in cell death in response to pathogen invasion, abiotic stresses and chemical elicitation, our data show that cell death induced by cadmium is also a form of programmed cell death mediated by caspase-like proteases. We have established a key role of hydrogen peroxide and calcium in cadmium-induced apoptotic cell death and have demonstrated that oxidative stress is associated with both cadmium and camptothecin-triggered cell death. We have also shown that polyamine spermine can effectively preserve the cell viability at conditions of chemical stress.

Table 1 (abstract S19) Effect of caspase peptide inhibitors, antioxidants (ascorbic acid, catalase, spermine) and calcium channel blocker $LaCl_3$on viability of CPT- or Cd-treated tomato suspension cells

Chemicals	Cell viability (%)
Control	97.5
CPT 5 μM	72.5
$CdSO_4$100 μM	65.0
CPT μM + Ac-YVAD-CMK 100 μM	92.5
$CdSO_4$100 μM + Ac-YVAD-CMK 100 μM	94.0
$CdSO_4$100 μM + Z-asp-CH2-DCB 100 μM	91.5
CPT 5 μM + ascorbic acid 100 μM	93.5
CPT 5 μM + catalase 10 Units/ml	91.5
CPT 5 μM + spermine 100 μM	88.5
$CdSO_4$100 μM + ascorbic acid 100 μM	92.5
$CdSO_4$100 μM + catalase 10 Units/ml	91.5
$CdSO_4$100 μM + spermine 100 μM	88.2
CPT + 5 μM + $LaCl_3$100 μM	95.0
$CdSO_4$100 μM + $LaCl_3$100 μM	94.5

Table 2 (abstract S19) Effect of ethylene and ethylene inhibitor AVG on cell viability of CPT and cadmium treated tomato cell suspension

Chemicals	Cell viability (%)
Control	95
CPT 5 μM	72
$CdSO_4$ 100 μM	68
AVG 10 μM	98
Ethylene (Eth) 100 μL/L	95
CPT 5 μM + Eth 100 μL/L	50
$CdSO_4$ 100 μM + Eth 100 μL/L	48
CPT 5 μM + AVG 10 μM	90
$CdSO_4$ 100 μM + AVG 10 μM	86
CPT 5 μM +AVG 10 μM + Eth 100 μL/L	38
$CdSO_4$ 100 μM + AVG 10 μM + Eth 100 μL/L	49

Ethylene was found to be an important mediator of plant cell death. The finding that ethylene greatly stimulated cadmium-induced cell death and that cadmium treatment enhanced endogenous ethylene production indicated that ethylene participates in cadmium-induced cell death in tomato suspension cells. The application of specific inhibitors of phospholipase C, phospholipase D, inositolphosphate monophosphatase, inositol-3-phosphate kinase and phosphatidic acid caused considerable decrease of Cd-stimulated cell death and are the first more detailed evidence that Cd-triggered cell death in plants involves the phospholipid pathway.

Collectively, the cell response to cadmium elicitation and the inhibitors indicate that Cd-triggered cell death is analogous to cell death in response to CPT treatment [1, 2, 3, 4] and involves caspase-like proteases, oxidative stress and ethylene. Cd-induced cell death in plant cells exhibits similarities to HR [5] and cell death induced by known apoptosis inducing chemicals and to its effect in animal systems.

Acknowledgements

Elena Iakimova and Veneta Kapchina-Toteva were supported by STSM grants (COST Action 844) and FP5 IHP LSF grants. Authors are thankful to France Harren and Luc-Jan Laarhoven for the assistance with the laser photoacoustics.

References

1. de Jong A, Yakimova E, Hoeberichts F, Maximova E and Woltering E: **Chemical-induced apoptotic cell death in tomato cells: Involvement of caspase-like proteases.** *Planta* 2000, **211:**656–662.
2. de Jong A, Yakimova E, Kapchina V and Woltering E: **A critical role of ethylene in hydrogen peroxide release during programmed cell death in tomato suspension cells.** *Planta* 2002, **214:**537–545.
3. Woltering E, van der Bent A and Hoeberichts F: **Do plant caspases exist?** *Plant Physiol* 2002, **130:**1764–1769.
4. Woltering E, de Jong A, Iakimova E, Kapchina V and Hoeberichts F: **Ethylene: Mediator of oxidative stress and programmed cell death in plants.** *Biology and Biotechnology of the Plant Hormone Ethylene III* IOS Press: Vendrell 2003, 315–323.
5. Iakimova E, Batchvarova R, Kapchina-Toteva V, Popov T, Atanassov A and Woltering E: **Inhibition of apoptotic cell death induced by *Pseudomonas syringae* pv. *tabaci* and mycotoxin Fumonisin B1.** *Biotech & Biotech Eq.* 2004, **18:**34–46.

S20

Effect of nitric oxide on concentration of intracellular free Ca^{2+} in transgenic *Arabidopsis thaliana* plants during oxidative stress

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BMC Plant Biology 2005, **5(Suppl 1):**S20

Background: In recent years exogenous NO was shown to (i) influence plant growth and development, (ii) take part in the response of plants to pathogens, light and ABA-induced stomatal closure, and (iii) reduce consequences of oxidative stress generated by treatment with herbicides [1, 2, 3, 4]. It is also known that a number of chemical and physical stimuli, including oxidative stress, mediates their effects *via* transient increases in the concentration of intracellular free Ca^{2+} ($[Ca^{2+}]_{cyt}$). Therefore, the aim of our work was to study the influence of exogenous NO on the increase of $[Ca^{2+}]_{cyt}$ induced by oxidative stress in plant cells with changes in $[Ca^{2+}]_{cyt}$ during oxidative stress being measured in *Arabidopsis thaliana* seedlings transformed to express apoaequorin.

Materials and methods: 8-day old seedlings of *A. thaliana* were incubated in coelenterazine (5 μM) diluted in methanol for 6 h to reconstitute the aequorin. Chemiluminescence measurements were performed with a digital chemiluminometer. The peak-value of the stimuli-induced $[Ca^{2+}]_{cyt}$ transient was calculated as a described by Cobbold and Rink [5] with some modifications [6]. Oxidative stress induced by hydrogen peroxide (10 mM) [7]. To evaluate a possible effect of NO on the increase $[Ca^{2+}]_{cyt}$ during the oxidative stress seedlings were pre-treated with NO donor NOR-1 (25×10^{-6} M). Oxidative stress resulted in increased $[Ca^{2+}]_{cyt}$ (See Figure 1). The single spike of chemiluminescence was observed after a lag-phase of 20–40 sec. After $[Ca^{2+}]_{cyt}$ was increased from 0.1 μM to 1.3 μM and in 1.5–2 min was returned to a basal level. Our data are in accordance with data obtained by Price et al [7].

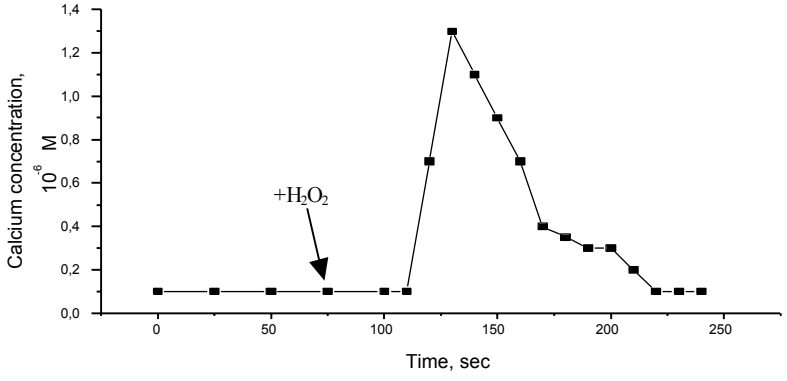
Results: Pre-treatment of seedlings with NOR-1 led to a single Ca^{2+}-spike (see Figure 2) similar to that observed during oxidative stress. However, the level of $[Ca^{2+}]_{cyt}$ was increased only to 0.7 MM. In this way, the increase in $[Ca^{2+}]_{cyt}$ was by 50% lower as compared with control ones. These data testify that NOR-1 interferes with $[Ca^{2+}]_{cyt}$ elevations caused by the oxidative stress in seedlings and that a potential communication point for cross-talk between signal transduction pathways (NO signal transduction and signal transduction during oxidative stress) is the $[Ca^{2+}]_{cyt}$.

Conclusion: These data suggest that NO plays an important role in the activation of plant defense responses after oxidative stress. It may partly explain that NO is able to inactivate directly the reactive oxygen species (ROS) [8]. The presence of an unpaired electrons within the NO molecule gives it its reactive species properties and is also the origin of its duality. At physiological concentrations NO may play a protective role acting as a chain inhibitor to limit the damage.

References

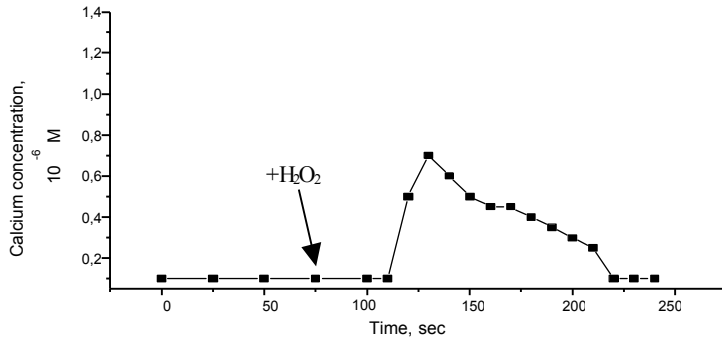
1. Delledonne M, Xia Y, Dixon RA and Lamb C: **Nitric oxide functions as a signal in plant disease resistance.** *Nature* 1998, **394:**585–588.
2. Beligni MV and Lamattina L: **Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants.** *Planta* 2000, **210:**215–221.
3. Neill SJ, Desican R, Clarke A and Hancock JT: **Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells.** *Plant Physiol* 2002, **128:**13–16.
4. Beligni MV and Lamattina L: **Nitric oxide protects against cellular damage produced by methylviologen herbicides in potasto plants.** *Nitric Oxide: Biol and Chem* 1999, **3:**199–208.
5. Cobbold PH and Rink TJ: **Fluorescence and bioluminescence measurments of cytoplasmic free calcium.** *Biochem J* 1987, **248:**313–328.
6. Knight H, Trewavas AJ and Knight MR: **Cold calcium signalling in Arabidopsis involves two cellular pools and change in calcium signature after acclimation.** *Plant Cell* 1996, **8:**489–503.
7. Price AH, Taylor A, Ripley SJ, Griffiths A, Trewavas AJ and Knight MR: **Oxidative signals in tobacco increase cytosolic calcium.** *Plant Cell* 1996, **6:**1301–1310.
8. Beligni MV and Lamattina L: **Is nitric oxide toxic or protective?** *Trends in Plant Sci* 1999, **4:**299–300.

Figure 1 (abstract S20)



Cytosolic calcium response of *Arabidopsis* seedlings under action of oxidative stress induced by H_2O_2

Figure 2 (abstract S20)



Effect of NOR-1 pretreatment on $[Ca^{2+}]_{cyt}$ response induced by oxidative stress

S21
Cytogenetic effect of herbicides on plant cells

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BMC Plant Biology 2005, **5(Suppl 1):**S21

Background: Herbicide application for weed control is an integral part of the method for chemical plant protection in agriculture. Herbicides are known to be used partially only for weed destruction (as to various assessments from 5 to 40% of applied preparation amount) and the rest of their amount remains in an environment and due to high stability is not able to undergo quick physicochemical and biological degradation. Being a factor of man's impact on the environment, herbicides can play a role of toxicants under certain conditions, with manifestation of their effect on different organisation levels of living organisms.

Methods: A comparative estimation of mutagenity for different commercial herbicide preparations was performed. Gesagard, granstar, zenkor, semerone, simazine and treflan were used for application on few plant test models such as *Allium cepa L.*, *Hordeum vulgare L.* and *Pisum sativum L.* Crop seeds were germinated in water solutions of respective herbicide preparations. Seeds germinated in distilled water were used as the control. Concentration of the substances under test were chosen experimentally with due account of recommended doses, their solubility, as well as the influence on seed germination.

Results: It was established that simazine caused an increase in the number of chromosome aberrations. Gesagard, zenkor, semeron and treflan affected to some or other extent cytoskeleton structures and chromosome disjunction during mitosis. Cells with apoptotic characters were detected in plants seedlings treated with herbicides (see Figure 1).

Herbicides observed by us under pesticide effect indicate that cells undergo a state of oxidative stress. Active oxygen forms (AOFs) emerging under oxidative stress are known to affect the cytoskeleton structure [1]. In their turn, disorders in the cytoskeleton result in a disorganisation of intracellular transport and oxygen consumption resulting in intracellular hyperoxia and the higher AOFs [2]. Disorders in the stability of cytoskeleton proteins under oxidative stress cause inhibition of mitotic events and resulted in cell death.

Conclusion: The cytogenetic effects of gesagard, zenkor, semeron, simazine and treflan were established. It was found that these herbicides are capable of destroying mitotic figures and delaying mitotic division which can result in plant cell death.

References

1. Egorova AB, Uspenskaya YuA, Mikhutkina SV and Stavitskaya EYu: **Damage of cytoskeleton and cell membranes in apoptosis.** *Adv Cur Biol* 2001, **121:**502–510.
2. Lyu BN: **An oxygen-peroxide concept of apoptosis and possible versions of its mechanism.** *Adv Cur Biol* 2001, **121:**488–501.

S22
Glutathione-dependent formaldehyde dehydrogenase/GSNO reductase from *Arabidopsis*. Expression pattern and functional implications in phytoremediation and pathogenesis

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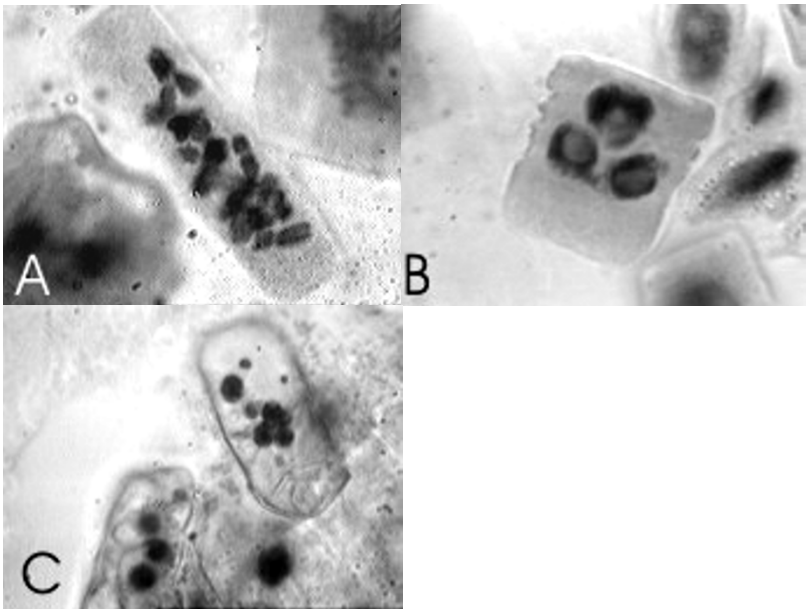
BMC Plant Biology 2005, **5(Suppl 1):**S22

The glutathione-dependent formaldehyde dehydrogenase (FALDH) is the main enzyme of the formaldehyde detoxification system in eukaryotes. In *Arabidopsis* FALDH is coded by a single gene, which is constitutively expressed [1]. By immunolocalization experiments on *Arabidopsis* root and leaf sections, we demonstrate that the pattern of expression of the enzyme is cell specific. By using tobacco BY-2 cell cultures we show that FALDH co-localizes with tubulin on the cortical microtubules and the microtubules figures (preprophase band, mitotic spindle and phragmoplast), which suggests a role for FALDH in some plant-specific function during cell division. Over-expression of FALDH in *Arabidopsis* plants results in a 25% increase in the efficiency of elimination of exogenous formaldehyde, whereas plants with reduced levels of FALDH, bearing antisense constructs, show a reduced ability and slower rate in formaldehyde

Figure 1 (abstract S21)



Cells of root meristem of *Hordeum vulgare* L. after treflan treatment (1 mg/l). **A**. k-metaphase; **B**. three-nucleus interphase; **C**. apoptotic cells.

elimination [2]. These results confirm the central role of FALDH in formaldehyde metabolism in plants and have important implications in the phytoremediation of environmental formaldehyde.

The importance of FALDH has been greatly increased by the discovery of its potent activity toward S-nitrosoglutathione, the condensation product of glutathione and nitric oxide (NO) [3, 4, 5]. NO and NO-related metabolites, such as S-nitrosothiols (SNOs) play a central role in signal transduction and host defense [6]. We have investigated the gene response to mechanical wounding and plant hormones involved in the signal transduction pathway, showing that the gene is down-regulated by wounding in a JA-dependent pathway, and that it is transcriptionally activated by salicylic acid [7]. This is the first time that regulation of FALDH in response to signals associated with plant defense has been demonstrated.

References

1. Martínez MC, Achkor H, Persson B, Fernández MR, Shafqat J, Farrés J, Jörnvall H and Parés X: ***Arabidopsis* formaldehyde dehydrogenase: molecular properties of plant class III alcohol dehydrogenase provide further insights into the origins, structure and function of plant class P and liver class I alcohol dehydrogenases.** *Eur J Biochem* 1996, **241:**849–857.
2. Achkor H, Díaz M, Fernández MR, Biosca JA, Parés X and Martínez MC: **Enhanced formaldehyde detoxification by overexpression of glutathione-dependent formaldehyde dehydrogenase from Arabidopsis thaliana.** *Plant Physiol* 2003, **132:**2248–2255.
3. Jensen DE, Belka GK and Du Bois GC: **S-Nitrosoglutathione is a substrate for rat alcohol dehydrogenase class III isoenzyme.** *Biochem J* 1998, **331:**659–668.
4. Liu L, Hausladen A, Zeng M, Que L, Heitman J and Stamler JS: **A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans.** *Nature* 2001, **410:**490–494.
5. Sakamoto A, Ueda M and Morikawa H: ***Arabidopsis* glutathione-dependent formaldehyde dehydrogenase is an S-nitrosoglutathione reductase.** *FEBS Lett* 2002, **515:**20–24.
6. Wendehenne D, Pugin A, Klessig DF and Durner J: **Nitric oxide: comparative synthesis and signaling in animal and plant cells.** *Trends Plant Sci* 2001, **6:**177–183.
7. Delledonne M, Xia Y, Dixon RA and Lamb C: **Nitric oxide functions as a signal in plant disease resistance.** *Nature* 1998, **394:**585–588.
8. Huang X, Stettmaier K, Michel C, Hutzler P, Mueller MJ and Durner J: **Nitric oxide is induced by wounding and influences jasmonic acid signaling in Arabidopsis thaliana.** *Planta* 2004, **218:**938–946.
9. Díaz M, Achkor H, Titarenko E and Martínez MC: **The gene encoding glutathione-dependent formaldehyde dehydrogenase/GSNO reductase is responsive to wounding, jasmonic acid and salicylic acid.** *FEBS Letters* 2003, **543:**136–139.

S23
Plant phenolic metabolites as the free radical scavengers and mutagenesis inhibitors

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BMC Plant Biology 2005, **5(Suppl 1):**S23

Plant secondary metabolites are involved in versatile functions on different levels in the plant organism. One of the roles is the scavenging of free radicals and the protection against excess

oxidation caused by UV irradiation, chemical oxidants or pathogen attack or other kinds of stress. Involved are phenolic compounds from different classes such as numerous phenol carboxylic acids, hydroxylated flavonoids such as flavones, flavonols, leucocyanidins, anthocyanins and procyanidins as well as isoflavonoids. Many of these substances have been isolated from plant species possessing valuable and intensively studied medicinal properties.

For assessment of the free radical scavenging and antioxidant capacity of phenolic complexes in plants the chemical *in vitro* (cell free) tests can be used for their relative simplicity and sometimes reasonable cost. Here, we describe the application of several antioxidant and anti-free radical spectrophotometric assays for testing the antioxidant abilities of some rarely studied plant species containing different classes of polyphenols. In addition, the antimutagenic bacterial assays were used to examine the in vivo genoprotective activity of these compounds against chemical mutagens. Among the investigated compounds there are lipophilic flavones and their glucuronides from *Scutellaria baicalensis* and Iridaceae-type isoflavonoids from *Belamcanda chinensis*. Phenolic acids, procyanidins and flavonols containing Lamiaceae species such as *Leonurus sp*, *Lamium sp*., *Stachys officinalis*, *Marrubium vulgare*, *Galeopsis speciosa* have been also studied to comprise wider spectrum of different types of polyphenolics.

The assays used address the different aspects of antioxidant properties such as: free radical scavenging in aqueous and non-aqueous environment (ABTS and DPPH colorimetric tests), scavenging of enzymatically generated superoxide anion radical, transition metal reduction ability by phosphomolybdenum complex formation [2], protection against hydroxyl radical induced polyunsaturated lipid peroxidation in the Fenton reaction system.

It is important to employ several antioxidant assays for each object as there are usually different mechanisms of the antioxidation involved that results in varying outcome depending on the test used. For example the polyphenolic mixture from *Stachys officinalis* showed the weakest potential in the DPPH discoloration test whereas was the strongest one in molybdate reduction assay what clearly indicates the complexity of the involved mechanisms.

The antimutagenic activity of the extracted phenol complexes and isolated compounds correlates with free radical scavenging. In the Ames bacterial assays [1] the direct mutagenesis by chemical mutagens can be distinguished from the mutagenesis induced by activation of pro-mutagen with cytochrome P-450 enzymatic fractions. The aglycones were clearly more efficient than glycosides in inhibition of mutagenesis, the lipophilic flavone from *Scutellaria baicalensis*–baicalein being the most efficient. Other flavonoids were effective in inhibition of indirect mutagenesis that can be attributed to the inhibitory action against the pro-mutagen activating enzymes [3].

Free radical scavenging by the low molecular weight compounds can play an important role as the last line of defense against oxidative damage of the cells for they are more stable than enzymatic antioxidant apparatus and can be easily accumulated in stress conditions (e.g deposited in the cell wall or the vacuole). Superoxide scavenging can protect the cells against the production of deleterious peroxynitrite upon reaction of the relatively harmless superoxide with an important signaling molecule–nitric oxide.

The activity of complex extracts is sometimes stronger than individual compounds, which can be interpreted as the necessity for preserving the native composition, more effective when acting in oxidation/reduction cascades and thereby able to reduce the formation of harmful oxidation end-products. The *in planta* function of the antioxidant and antigenotoxic compounds should be further explored in order to obtain the complete insight into their role in protecting the plant cell.

References

1. Maron D and Ames BN: **Revised methods for the *Salmonella* mutagenicity test.** *Mutat Res* 1983, **113:**173–215.
2. Prieto P, Pineda M and Aquilar M: **Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of vitamin E.** *Anal Biochem* 1999, **269:**337–341.
3. Wozniak D, Lamer-Zarawska E and Matkowski A: **Antimutagenic and antiradical properties of flavones from the roots of *Scutellaria baicalensis* Georgi.** *Nahrung* 2004, **48:**9–12.

S24

Lowered stomata conductance promotes the oxidative burst, an essential factor in the promotion of programmed cell death

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BMC Plant Biology 2005, **5(Suppl 1):**S24

The role of stomata in photosynthesis and their importance in plant productivity is well established and a number of studies have shown their importance for the plant's interaction with the abiotic environment. However an increasing amount of evidence is pointing toward an important role for stomata also in biotic defense responses. Using programmed cell death mutants and pathogen infection we set out to investigate the role of stomata in biotic stress. Physiological characterization of the *lsd1* mutant of *Arabidopsis thaliana* revealed a correlation between stomata conductance, H_2O_2 production and the spreading of cell death. When challenging wild type plants with the bacterial pathogen *Pseudomonas syringae*, stomata closed and limiting gas exchange strongly enhanced the spreading of cell death both in infected plants and in the *lsd1* mutant. Further studies showed that limiting gas exchange enhances the production of reactive oxygen species that lead to the formation of focused hypersensitive response like lesions in ws-0. Our results clearly indicate the importance of stomata regulation in the spreading of cell death.

S25

Nitric oxide in cell damage and protection

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BMC Plant Biology 2005, **5(Suppl 1):**S25

Less than ten years ago, the impact on plant biology of the gaseous free radical gas nitric oxide ($NO^{\cdot}$) related only to its toxic effects as a component of NO_x, released into the atmosphere as an air

pollutant during the combustion of fossil fuels. It is now clear that NO is a multi-faceted and versatile endogenous signalling molecule with an importance in many if not all aspects of plant growth and development. At least three enzymatic sources of NO in plants have been characterised and mechanisms that serve to scavenge NO have also been identified. Downstream signalling responses to NO include generation and action of the second messenger molecules calcium, cyclic GMP and cyclic ADPR, protein phosphorylation, protein nitrosylation and specific effects on gene expression. NO also interacts directly with Reactive Oxygen Species (ROS) and with components of ROS-activated signalling pathways. NO and ROS play key roles in an orchestra of plant defence responses. Rapid generation of NO and ROS following pathogen or elicitor challenge mediates a multitude of metabolic and transcriptional alterations including Programmed Cell Death (PCD). However, it is important to note that in some cases the actions of NO can be cytoprotective rather than toxic, potentially via antioxidant effects of NO. Furthermore prevention of NO synthesis or action can delay or inhibit PCD. In addition to the roles of NO and ROS in biotic stress responses, NO and ROS generation also occurs in response to various abiotic stresses, including UV radiation which itself can induce PCD. Recent data suggest that NO mediates some UV responses and that UV radiation can also stimulate the release of NO_x from leaves. Key research questions to be addressed must be directed to the effects of UV on NO generation and action in plants. Research programmes will require methods to assess accurately NO emissions from leaves and other organs and to determine NO concentrations in cells and sub-cellular microdomains; the use of mutants and transgenic plants altered in NO synthetic and scavenging capacities; analyses of the molecular and biochemical events required for activation of PCD by NO and UV; and the development of techniques to monitor simultaneously cell death, NO and ROS generation in the field during exposure to UV.

S26
Nitrotyrosination of plant α-tubulin: potential mechanisms of influence on cellular processes

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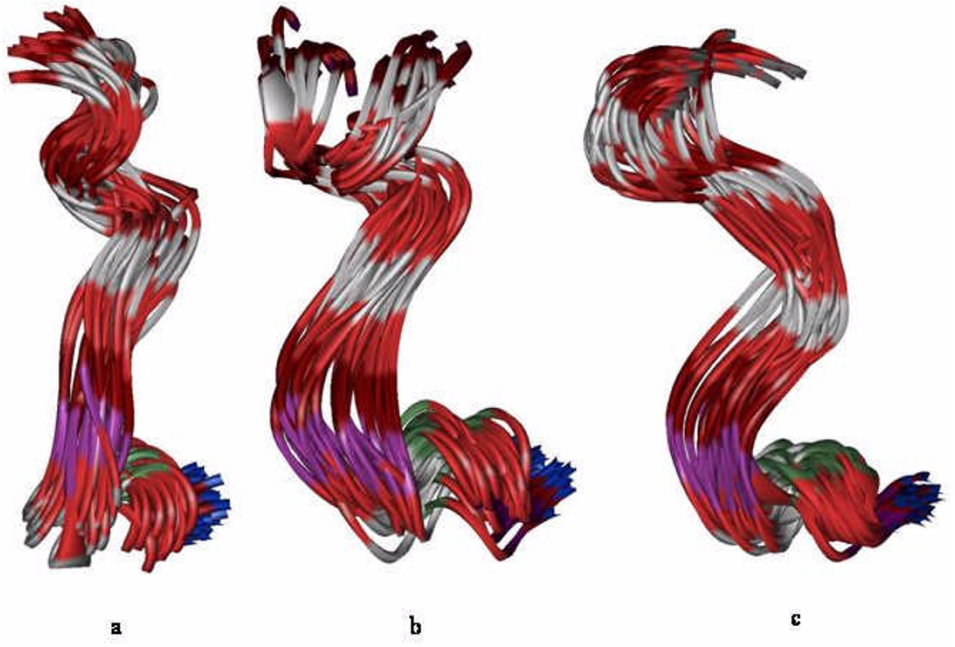
BMC Plant Biology 2005, **5(Suppl 1):**S26

3-Nitrotyrosine, the product of interaction between the free tyrosine with nitric oxide, is characterized by its posttranslational incorporation into carboxyl terminus of α-tubulin molecule via tubulin-tyrosine ligase, similar to unmodified tyrosine residue. However, α-tubulin C-terminal nitrotyrosination is irreversible unlike an enzymatically regulated tyrosination/detyrosination cycle. The nitrotyrosination of α-tubulin results in an appearance of some changes in animal cell morphology and their microtubule organization, a loss of some cellular functions, and in intracellular redistribution of cytoplasmic dynein. Since, nitrotyrosination in plant cell has not been investigated, the aim of our study was to reconstruct the spatial structure of nitrotyrosinated plant α-tubulin with following modeling of possible mechanisms of this posttranslational modification influence on cellular processes.

Figure 1 (abstract S26)



Modelling of different allowed conformation of C-terminal tails of α-tubulin from goosegrass: **A.** Detyrosinated molecule; **B.** Tyrosinated molecule; **C.** Nitrotyrosinated molecule

The last 10–12 amino acid residues of α-tubulin C-terminal region, unlike the main part of the molecule, form a free oscillating tail that can be considered as individual structural domain. The results of reconstructing the spatial structure of detyrosinated, tyrosinated and nitrotyrosinated goosegrass (*Eleusine indica* (L.) Gaerth.) α-tubulin forms and studies of their behavior testify that these modifications change substantially the level of mobility of flexible α-tubulin C-terminal domain. We found that tyrosination increases substantially the mobility of C-terminus (see Figure 1).

The root mean square deviation (RMSD) in coordinates of amino acid residues composing this region grows from 0.48 A for detyrosinated form, to 0.55 A for tyrosinated α-tubulin as was calculated on the basis of molecular fluctuations trajectory in 500 ps interval. These results correlate with data about predomination of detyrosinated α-tubulin in long-time living microtubules and *vice versa*. The breakdown of tyrosination/detyrosination cycle due to incorporation the nitrotyrosine into C-terminus should resulted in predomination of short-time living microtubules over long-time living microtubules. It is necessary to note that mobility of nitrotyrosinated terminus is lower than the tyrosinated one, but it is higher than the detyrosinated terminus. In the last case RMSD is 0.52 À. It can lead to the appearance of microtubules with a certain "intermediate" lifetime and, as a consequence, modify the microtubules organization in cells.

It is known that the tubulin C-terminal region is responsible for interactions with structural and motor MAPs. In combination with the above mentioned data about the redistribution of cytoplasmic dynein after nitrotyrosination of α-tubulin we can speculate that nitrotyrosination can influence processes of tubulin-MAP recognition and interaction. This assumption is confirmed by our results on modeling the interaction of α-tubulin with the MT-binding domain of dynein heavy chains. The high content of dicarboxylic amino acid residues, especially glutamate residues, is the hallmark of the flexible C-terminal region of both tubulin subunits. It has to be correlated with the necessity of presence, a surface enriched with diamidate amino acid residues in the dynein MT-binding domain structure. The surface of dynein MT-binding domain formed by residues Lys3148, Lys3151, Lys3154, Lys3155, Arg3162, Lys3287, Lys3300, Arg3327, Lys3328 is correspondent to this factor. The surface is flanked by residues Asp3144, Asp3146 and Asp3320, Asp3321 on both sides. Included in nitrotyrosine nitro-group possesses an additional negative charge, that is not typical for dynein-tubulin interaction, and locates close to flanking residues. This location results in a strong decrease in the affinity between the dynein contact surface and α-tubulin C-terminal region.

S27
Nitric oxide, a signaling molecule in plant cell reactivation

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BMC Plant Biology 2005, **5(Suppl 1):**S27

Nitric oxide is known to act as a biological messenger in divers signal transduction pathways in animal organisms. Initial investigations suggest that plants use nitric oxide as a signaling molecule via pathways remarkably similar to those found in mammals. Especially, the siganiling role of NO during plant defense reactions is well established. However, mounting evidences support the hypothesis that NO is a more general effector of plant growth and development.

In our laboratory, alfalfa cell cultures were used to investigate the possible involvement of NO in the regulation of cell division and differentiation in plant cells. The homogenous population of leaf protoplasts were cultured in the presence of a NO donor, sodium nitroprusside (SNP) and/or an inhibitor, NG-monomethyl-L-arginine (L-NMMA). BrdU incorporation frequency into the nuclei of the protoplast-derived cells indicated that the entry into the S-phase of the cell cycle is enhanced by SNP and inhibited by L-NMMA treatments, respectively (see Figure 1). Experiments have also been carried out with continuously dividing cell suspension cultures. The obtained data indicated that these type of cells are insensitive to similar treatments shown to affect protoplast-derived cell division (see Figure 2).

In addition to cell cycle progression, the effect of the above drugs on the auxin-induced formation of embryogenic competent cells from leaf protoplasts (for more details see [1, 2]) have been followed. The promotive effect of SNP and the inhibitory effect of L-NMMA have been observed on the process, especially at low exogenous auxin (0.02 μM 2,4-dichlorophenoxyacetic acid) supplementation.

Conclusion: The obtained experimental data indicated that NO is required for and can stimulate auxin-mediated activation of cell division as well as embryogenic cell formation from differentiated leaf cells (protoplasts), but is not involved in the regulation of cell cycle progression of continuously dividing cells.

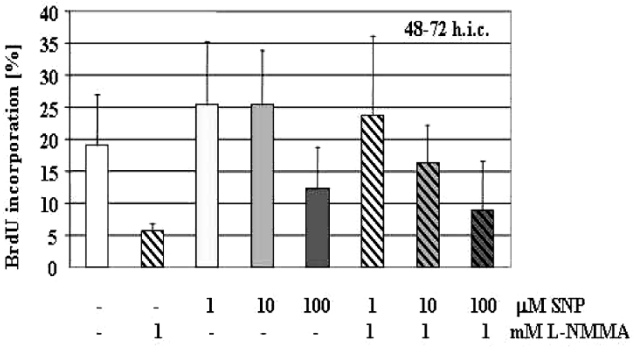
Acknowledgements

The presented work was partially supported by the national grant OTKA T34818.

References

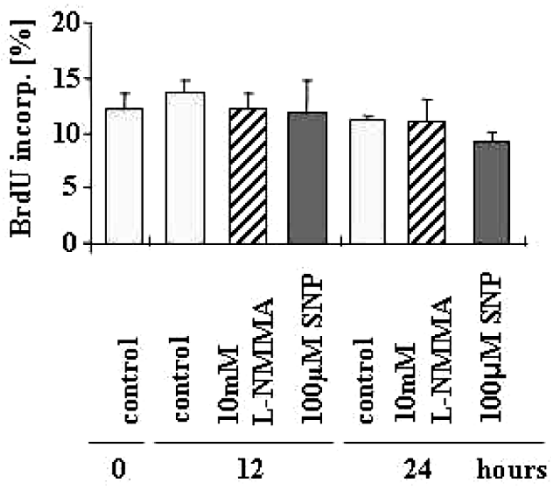
1. Feher A, Pasternak TP and Dudits D: **Transition of somatic plant cells to an embryogenic state.** *Plant Cell Tissue and Organ Culture* 2003, **74:**201–228.

Figure 1 (abstract S27)



BrdU incorporation frequency into the nuclei of leaf protplast-derived alfalfa cells during the third day of culture. The cells were treated by the indicated drugs affecting endogenous NO formation. For cell culture details see [2].

Figure 2 (abstract S27)



BrdU incorporation frequency into the nuclei of cell suspension-cultured alfalfa cells during the third day after subculture. The cells were treated by the indicated drugs affecting endogenous NO formation. For cell culture details see [2].

2. Pasternak T, Prinsen E, Ayaydin F, Miskolczi P, Potters G, Asard H, Van Onckelen H, Dudits D and Fehér A: **The role of auxin, pH and stress in the activation of embryogenic cell division in leaf protoplast-derived cells of alfalfa (Medicago sativa L.).** *Plant Physiol* 2002, **129:**1807–1819.

S28

Alteration in ascorbate and ascorbate peroxidase in programmed cell death and oxidative stress

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BMC Plant Biology 2005, **5(Suppl 1):**S28

Different stress conditions, of biotic and abiotic nature, enhance the cellular production of reactive oxygen species (ROS) [1]. Due to their reactive nature, ROS are potentially harmful to all cellular components. Apart their destructive nature, ROS behave as metabolic regulators, being considered as secondary messengers. Indeed, ROS can trigger pathways aimed at saving cells from demise; however, under certain conditions, they can also impair the cellular red/ox balance as to activate a programmed cell death (PCD) process [2, 3]. These differences in the ROS-dependent responses seem to be due to different localization, timing and level of ROS production under different stimuli [4, 5]. Moreover, the possibility that the co-production of other reactive species is a critical point for the activation of different defence responses has been recently underlined. Increasing attention has been paid to nitric oxide (NO) as a signal molecule synergically acting with ROS in the activation of PCD [6, 7]. The level of ROS and the cellular redox homeostasis are regulated by different antioxidant systems; among these, ascorbate (ASC) plays a pivotal role, it being both a direct scavenger of ROS and the electron donor of ascorbate peroxidase (APX), a key enzyme for scavenging hydrogen peroxide in plant cells [8, 9].

Here we report data showing that in tobacco cultured cells a moderate oxidative stress did not lead to apoptotic or necrotic events whereas, when H_2O_2 production was increased over a certain range of concentration, an induction of cell death with the features of necrosis was achieved. On the other hand, when the tobacco cells were simultaneous treated with NO and H_2O_2 generators a PCD program was triggered. The scavengers ASC and APX change differently under the various stress conditions. During the moderate oxidative stress a transient increase in APX activity occurred whereas, in the induction of cell necrosis, the activity of APX decreased proportionally to cell death. Under such conditions, no alteration in the APX gene expression was evident and 24 hours after the generation of the oxidative stress, APX activity was significantly increased in the surviving cells, in order to overcome the oxidative stress and to avoid further cell death. When the PCD program was triggered in these cells by the contemporary increase of NO and H_2O_2 the suppression of APX occurred both at the translation or post-translation level. The decrease in APX seems to be one of the first alteration in the redox regulating systems induced in the plant cells in route to PCD.

References

1. Dat J, Vandenabeele S, Vranovà E, Van Montagu M, Inzè D and Van Breusegem F: **Dual action of the active oxygen species during plant stress responses.** *Cell Mol Life Sci* 2000, **57:**779–795.
2. Lam E, Kato N and Lawton M: **Programmed cell death, mitochondria and the plant hypersensitive response.** *Nature* 2001, **411:**848–853.
3. Vacca RA, de Pinto MC, Valenti D, Passarella S, Marra E and De Gara L: **Production of reactive oxygen species, alteration of cytosolic ascorbate peroxidase and impairment of mitochondria metabolism are early events in heat shock-induced programmed cell death in tobacco bright-Yellow 2 cells.** *Plant Physiol* 2004, **134:**1100–1112.
4. Mittler R: **Oxidative stress, antioxidants and stress tolerance.** *Trends Plant Sci* 2002, **7:**405–410.
5. De Gara L, de Pinto MC and Tommasi F: **The antioxidant systems vis à vis reactive oxygen species during plant-pathogen interaction.** *Plant Physiol Biochem* 2003, **41:**863–870.
6. Delledonne M, Xia Y, Dixon RA and Lamb C: **Nitric oxide functions as a signal in plant disease resistance.** *Nature* 1998, **394:**585–588.
7. de Pinto MC, Tommasi F and De Gara L: **Changes in the antioxidant systems as part of the signaling pathway responsible for the programmed cell death activated by nitric oxide and reactive oxygen species in tobacco Bright-Yellow 2 cells.** *Plant Physiol* 2002, **130:**698–708.
8. Smirnoff N: **Ascorbic acid: metabolism and functions of a multi-facetted molecule.** *Curr Opin Plant Biol* 2000, **3:**229–235.
9. Noctor G and Foyer CH: **Ascorbate and glutathione: keeping active oxygen under control.** *Annu Rev Plant Physiol Plant Mol Biol* 1998, **49:**249–279.

S29
Does NO participate in cytokinin signaling?

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BMC Plant Biology 2005, **5(Suppl 1):**S29

Background: Nitric oxide (NO) is a unique ubiquitous molecule in animals and plants [1]. It has been demonstrated that NO mediates ABA-induced stomatal closure and interferes with ethylene during the maturation and senescence of plant tissues. Recently published data [2, 3] suggested that NO is also involved in cytokinin signaling. However the above-mentioned data did not provide defenitive proof of a role of NO in cytokinin signaling. In order to obtain more conclusive evidence we have performed a study using P*arr5*::*GUS* transgenic *Arabidopsis* plants which express the *GUS* reporter gene under the transcriptional control of the cytokinin-responsive *ARR5* promoter. Additional experiments were also performed using *Amaranthus* seedlings.

Materials and methods: The *Arabidopsis* and *Amaranthus* assay systems were described in detail earlier [4, 5]. The cytokinin effects were measured quantitatively in both systems. For RNA blot analysis, 3-day-old Arabidopsis seedlings were incubated with BA (with oor without tested compounds) for 35 min. Total RNA was isolated and separated in a 1.5% agarose-formaldehyde gel, transferred to nylon membrane (Amersham) and hybrydized with radioactive-labeled DNA probe. For radioautography blots were exposed with BioMax MS films (Kodak) for 1–3 days. Quantification of signal was done on an integrative densitometer CD-50 (Desaga). As a control for loading, the blot was rehybridized with an *actin 2* gene probe.

Results: In our experiments, a strong competitive inhibitor of all three isoforms of animal NO synthase (NOS), L-NMMA, inhibited the accumulation of GUS activity in transgenic *Arabidopsis* harboring the *GUS* gene driven by a cytokinin-sensitive *ARR5* promoter. In accordance with earlier publication [ ], this inhibitor also suppressed cytokinin-dependent betacyanin accumulation in *Amaranthus*. In the *Arabidopsis* assay system, the effectiveness of L-NMMA was higher than in *Amaranthus* one: 1–2 mM L-NMMA inhibited cytokinin-induced effect in *Arabidopsis* at about 80–90%. In *Amaranthus*, similar inhibition was observed with 5 mM L-NMMA.

In addition to L-NMMA we have used its D-isomer (D-NMMA) which does not affect animal NOS. Experiments with L- and D-isomers have shown that both isomers were able to inhibit cytokinin action with similar effectiveness. Thus no functional difference between the active (in animals) L-form and its inactive D-analog was revealed in the *Arabidopsis* assay system.

To obtain more direct evidence, we treated *Arabidopsis* seedlings with NO. *Arabidopsis* seedlings were incubated in the presence of the NO donors NOR3 or SNAP. In our experiments neither SNAP nor NOR3, both tested in a wide concentration range, caused a cytokinin-like effect, namely the enhancement of GUS activity. This result argues against a role for NO as a direct messenger of the cytokinin signal. However, it does not exclude the possibility that NO has a role in a some parallel transduction pathway, which could be indispensable to effective cytokinin signaling. This possibility was tested experimentally by adding NO donors to *Arabidopsis* plants which were treated with BA and L-NMMA. However experiments showed that NO did not alleviate the L-NMMA inhibition of cytokinin-induced GUS activity. This result also does not support the participation of NO in cytokinin signal transduction to primary response genes, at least in *Arabidopsis*.

Next we explored whether L-NMMA inhibits cytokinin signaling at an early stage (before gene activation) or acts posttranscriptionally. To this end we analysed the steady state levels of *GUS* transcripts 35 min after cytokinin treatment of *Arabidopsis* seedlings. Results demonstrated that with no cytokinin treatment *GUS* gene expression was very low, the radioactive signal being hardly detectable. 35 min after cytokinin treatment a more than 30-fold increase of the *GUS* transcript was detected. 5 mM L-NMMA, which strongly inhibits the cytokinin-induced accumulation of GUS activity, had no influence on the cytokinin-induced accumulation of *GUS* transcripts. This result shows that L-NMMA acts posttranscriptionally.

Conclusion: Together the obtained results suggest that NOS inhibitor acted after the cytokinin signal transduction stage and NO has no direct role in eliciting the primary cytokinin response in plants, at least on cytokinin primary response genes in *Arabidopsis*.

Acknowledgements
This work was supported by grants of the Deutsche Forschungsgemeinschaft (Schm 814/13-1), the Russian Foundation of Basic Research (NN 01-04-04002/03-04-04001/04-04-49120) and INTAS N 01-0602.

References

1. Wendehenne D, Pugin A, Klessig DF and Durner J: **Nitric oxide: comparative synthesis and signaling in animal and plant cells.** *Trends Plant Sci* 2001, **6:**177–183.
2. Scherer GFE and Holk A: **NO donors mimic and NO inhibitors inhibit cytokinin action in betalaine accumulation in *Amaranthus caudatus*.** *Plant Growth Regul* 2000, **32:**345–350.
3. Tun NN, Holk A and Scherer GFE: **Rapid increase of NO release in plant cell cultures induced by cytokinin.** *FEBS Lett* 2001, **509:**174–176.
4. Romanov GA, Getman IA and Schmülling T: **Investigation of early cytokinin effects in a rapid *Amaranthus* seedling test.** *Plant Growth Regul* 2000, **32:**337–344.
5. Romanov GA, Kieber JJ and Schmülling T: **A rapid cytokinin response assay in *Arabidopsis* indicates a role for phospholipase D in cytokinin signalling.** *FEBS Lett* 2002, **515:**39–43.

S30
Reconstruction of the UV-time series weighted for the plant action spectrum based on the UV and total ozone data collected at Belsk, Poland in the period 1992–2003

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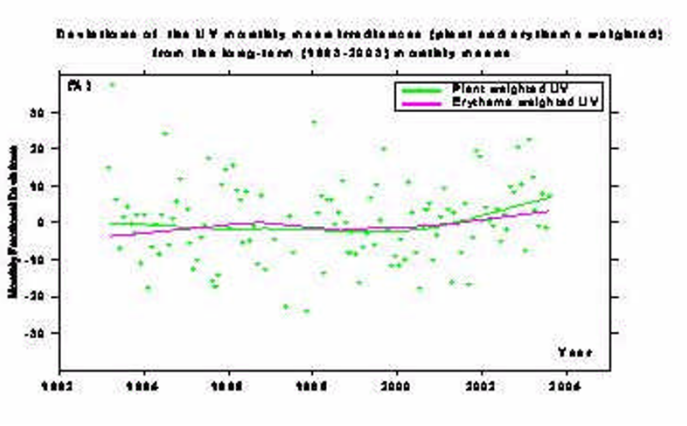
BMC Plant Biology 2005, **5(Suppl 1):**S30

The UV observations weighted for the plant action spectrum are rather limited. The activities in the UV ground-based measurements mainly focused on the human erythemal effects. The objective of this work is to find the long-term variations in the UV radiation weighted by the plant effects using the data (erythemal UV irradiance and total

Figure 1 (abstract S30)



The retrieved time series of the monthly fractional deviations of the UV radiation weighted by the plant action spectrum

ozone measurements) collected at Belsk (52N,21E), Poland, in the period 1992–2003.

The transfer function from the erythemal weighted UV radiation to the UV radiation convolved with the plant action spectrum is constructed using total ozone observations (from the Brewer spectrophotometer) and UV spectral and UV erythemal data (from the Brewer spectrophotometer and the broadband UV biometer SL 501 for the erythemal effects) taken during the intercomparison campaign of the spectrophotometers in Warsaw, May 2004. The long-term variations in the reconstructed data are discussed concerning the impact of total ozone and cloudiness fluctuations on the UV radiation for the period 1992–2003.

Conclusion: Reasonable reconstruction of the UV irradiance weighted by the higher plants action spectrum is possible if the erythemal UV irradiance and total ozone data are available. The erythemal irradiance is measured for many European sites by various broadband instruments. Thus, for many stations having also total ozone data (ground-based or from satellite observations) we can calculate the UV irradiance weighted by various biological effects.

References

1. Flint SD and Caldwell MM: **A biological spectral weighting function for ozone depletion research with higher plants.** *Physiol Plant* 2003, **117:**137–144.

S31

Analysis of proteins involved in programmed cell death in *Hemerocallis* petals

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BMC Plant Biology 2005, **5(Suppl 1):**S31

Discovery of characteristic proteins expressed by a given cell type and how these proteins change during development or pathology, has only been feasible very recently. Powerful separation techniques coupled with the sensitivity and selectivity of mass spectrometry, are used to identify these proteins. Computer-based, bioinformatics techniques are used to match unique peptides to the genome.

We recently received a Major Research Instrumentation award from the National Science Foundation (USA) to obtain new state-of-the-art protein analysis equipment. Specifically, we have acquired a research grade hybrid quadrapole ESI-MS/MS mass spectrometer interfaced with a capillary liquid chromatograph (cap-LC). This system (Micromass V-QTM) is a sensitive (femtomole), high resolution, time of flight mass spectrometer (Tof) with electrospray ionization (ESI) sample introduction interface for the capLC.

Tandem quadrapole mass spectrometric capabilities and high resolution permits the determination of amino acid sequence of peptides. Software associated with the spectrometer is used to unsort multiple charged versions of the same peptides and proteins, drive the LC separations, and handle other, expected complications in data analysis.

As part of this proposal, we received funds to develop an education and outreach module for educators. The proposed module involves proteomics-based experiments conducted for the study of cell death in plants. The daylily flower (*Hemerocallis* spp.) provides an ideal model system for the study of non-stress related programmed cell death in plants. The entire genetic program/cascade occurs over a compact 24-h period and can be studied in a series of specific sequential steps. We are currently analyzing changes in the daylily petal proteome during the various stages of cell death begining at 12 h prior to flower opening and ending at the final stages of cell death 12 h after opening. A shotgun proteomics approach is currently being utilized. Data derived from this study will not only provide valuable insight in programmed cell death in plants, but also be used to teach proteomics and the study of biological processes to educators involved in biological instruction.

S32

Uv induced ds(ss)-DNA damage: optical and electrical recognition

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BMC Plant Biology 2005, **5(Suppl 1):**S32

The main targets for UV irradiation in biological cells are DNA molecules [1]. Photochemical reactions in DNA caused by UV irradiation are the general processes that we use, in cell biology, to study the DNA damaged area. We analyzed:

• known mechanisms of UV induced DNA damage in cells: excited by UV light, nucleotide bases begin to take part in photochemical reactions of cyclobutane-pyrimidine dimmer and (4–6) adducts formation.

• absorption DNA spectrum behaviour in the range 200 – 300 nm changes of absorption by bases under UV irradiation.

• photoluminescence charge transport behaviour of ds-DNA molecules in wet, dry adsorbed layers with bases changes under UV irradiation.

Then the experimental investigations were carried out on 2 mM plant ds-DNA polymerized molecules (Eris, France [11]) that were dissolved in 3 mM NaOH buffer and 2 mM ss-DNA with 15 oligonucleotides bases: 3'-CCA CCG CTG CTG AGG-5', length 5,4 nm, wide 1 nm (Jena BioScience, Germany) were dissolved in 100 mM carbonate/bicarbonate buffer [2]. We prepared ds-DNA polymerized molecules water solutions dissolving 0,1 ml of DNA in buffer solution in 1, 40, 50 ml of water showed a pH of 7,4 (the volume ratios are 1:1, 1:40, 1:50, correspondingly) and ss-DNA water solution dissolving 1 ml of DNA in buffer solution in 5 ml of water showed pH of 7,4. The solutions in quartz cuvettes were irradiated by UV ($\prod$ = 200–400 nm) during 5–90 min with the light power 10^{20} photon/(cm^2 s).

From absorption spectra of different concentration of ds(ss)-DNA molecules in water solution the influence of UV irradiation on ds(ss)-DNA molecule absorption were revealed.

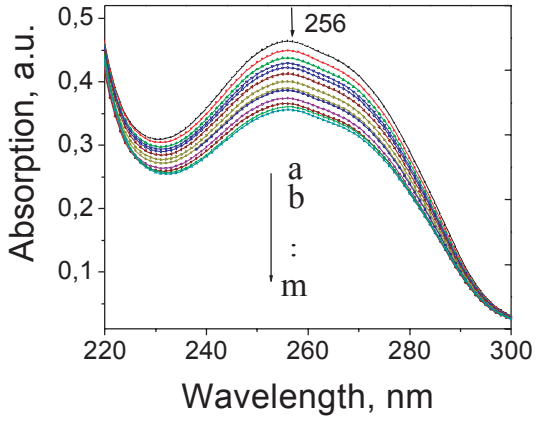
Photochemical reactions in the nucleotide base pairs with formation of dimmers and/or (4–6) adducts, that lead to the change of electronic levels structure of ds-DNA molecule and were revealed in absorption spectra (this spectrum is similar to presented in Figure 1): absorption maximum at 265 nm (4.7 eV) shifts to 269 nm (4.6 eV) – this caused by additional absorption on formed dimmers; maximum at 285 nm (4.3 eV) is absent – absorption on cytosine base becomes minimal: we assume that cytosine was used in dimmer formation.

The dependence of ds-DNA absorption intensity (for wavelengths 252 nm) on UV radiation time is represented in Figure 2b. Almost all changes in absorption take place during first 30 min of UV radiation, in the comparison with ds-DNA it could be caused by smaller number of adjacent T and C in ss-DNA bases sequence. The band in short wavelengths (λ < 235 nm) with maximum at 220 nm is not sensitive to UV irradiation as and for ds-DNA. These results prove that the UV effect in DNA becomes apparent in the excitation of nucleoside bases (absorption maxima is 235–280 nm) and photochemical reactions in DNA chains.

Photoluminescence spectra of wet dsDNA layer with networks have maxima at 432, 440 and 454, 463 nm before and after UV irradiation with 337 nm and 365 nm during one hour, respectively. The presence of photochemical reactions appear in the decreasing of photoluminescence intensity in this layer. Then it is possible intensive emission with participation of electronic states corresponds to molecular orbital systems in the nucleotide base pairs and sides of the ladder having a periodic structure with alternating sugar and phosphate groups. A part of the electron levels that determine photoluminescence spectrum can correspond the formation of dsDNA molecular networks.

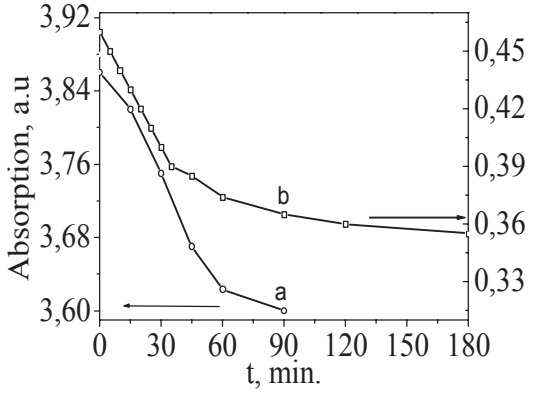
The presence of the photochemical reactions appear in the decreasing of dry absorbed ds-DNA molecular layer with networks conductivity under periodical switched UV irradiation: as a result the conductivity decreases after first radiation reflecting the reducing of pyrimidine bases (that formed dimmers) contributing to ds-DNA conductivity. The increase of the conductivity value after UV

Figure 1 (abstract S32)



Absorption spectra of ss-DNA molecules in water solution in the range 220—300 nm before (a) and after UV irradiation: b, c,..m during 5, 10.... 180 min correspondingly.

Figure 2 (abstract S32)



Absorption intensity of: a — ds-DNA molecules at 252 nm; b — ss-DNA molecules at 256 nm versus UV irradiation time.

irradiation could be caused by the particular reparation of ds-DNA under applied voltage of 1 V.

Conclusion: UV induced ds(ss)-DNA damage in buffer solution and in wet, dry absorbed layer, using optical and photoluminescence spectroscopy, as well as conductivity was revealed. Models of UV induced ds(ss)-DNA damage were accounted in the photochemical reactions of cyclobutane-pyrimidine dimmer and (4–6) adduct formation were used to recognise ds(ss)-DNA damage in their absorption spectra and wet ds-DNA damage in photoluminescence spectra and in the behaviour of conductivity under periodical switched of UV irradiation.

But it's important to develop offered methods for its further ability to direct evident possible photochemical reaction that took place in ds (ss)-DNA.

References

1. Courcelle J, Crowley DJ and Hanavalt PC: **Recovery of DNA replication in UV-irradiated *Escherichia coli* requires both excision repair and RecF protein function.** *J Bacteriol* 1999, **54:**916–922.
2. Csaki A, Kaplanek P, Moller R and Fritzsche W: **The optical detection of individual DNA-conjugated gold nanoparticle labels after metal enhancement.** *Nanotechnol* 2003, **14:**1262–1268.

S33

The effect of UV light on division capacity of *Helianthus mollis* L. protoplasts

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BMC Plant Biology 2005, **5(Suppl 1):**S33

Cultivated sunflower (*Helianthus annuus* L.), together with soybean, oil seed rape and peanut is one of the four most important crops for oil production and has a cultivated area that range from Russia, Eastern and Central Europe to Central America.

Wild sunflower species served as genetic base for the modern sunflower. Cultivated sunflower was selected from a very narrow genetic base. Enlarging the genetic base will allow it to be better adapted to environmental conditions. Many wild species are used to introduce useful traits, such as disease resistance (*Phomopsis* stem cancer, *Sclerotinia* wilt), cytoplasmic male sterility and increased oil content to the cultivated crop by introgression. Sexual hybridisation is limited in most cases to cultivars within a species or at best to a few wild species closely related to a cultivated crop. Species barrier thereby limits the genetic improvement by classic breeding. Somatic hybridisation, or somatic cell fusion technique, leading to the formation of viable cell hybrids is one of the methods used to overcome sexual incompatibility. In order to eliminate the unfavourable wild species traits in the somatic hybrids, a number of back crossings to the cultivated sunflower would be necessary. A method to reduce the backcrossings by reducing the amount of DNA from wild species in the hybrids, is the asymmetric fusion of the protoplasts. This technique is used to introduce fragments of the nuclear genome from the wild species into the complete genome of cultivated ones. A fragmentation of the donor genome can be induced by UV irradiation of the donor protoplasts prior to protoplast fusion.

The effect of UV-light treatment was determined on the basis of *Helianthus mollis* protoplast viability and division capacity. The isolation and purificartion of protoplasts was according to Krasnyanski and Menczel [1] protocol. The suspension of purified protoplasts was placed under a UV lamp during 60 min (wavelength of UV irradiation was 254 nm). Irradiation intensity was 2 Mmol m^{-2} s^{-1}. Protoplast viability was determined every 5 min, on 100 protoplasts, using FDA, under fluorescent microscope. The protoplasts were cultured in agarose droplets in VKM media. Plating efficiency (the number of cells dividing of total number of cells) was determined at 4 and 8 days in culture.

We observed the UV irradiation affects both, viability and division capacity of *H. mollis* protoplasts. Efficiency of UV-light treatment depends on its intensity and duration, a treatment in our conditions of more than 10 min decreases significantly the viability of the tested cells.

References

1. Krasnyanski S and Menczel L: **Production of fertile somatic hybrid plants of sunflower and *Helianthus giganteus* L. by protoplast fusion.** *Plant Cell Rep* 1995, **14:**232–235.

S34

Structural analysis of tobacco BY-2 cells treated with concerted synthesis of nitric oxide and hydrogen peroxide

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BMC Plant Biology 2005, **5(Suppl 1):**S34

Background: Nitric oxide (NO) is an important signalling molecule in plants. It influences many physiological processes [1]. During biotic stress plants produce NO and reactive oxygen species (ROS) simultaneously. The ratio of steady state concentrations of NO and ROS is decisive for the induction or prevention of cell death [2]. This effect was also demonstrated using artificial donor systems to generate NO and ROS (e. g. H_2O_2) [2, 3]. The aim of our work was to verify the specific effect of sodium nitroprusside (SNP; NO donor) and glucose with glucose oxidase (GGO; H_2O_2 donor) on the cell death process.

Materials and methods: Cell suspensions of tobacco (BY-2) were treated by 0.5 mM SNP, 0.5 mM potassium ferricyanide (PFC) as an analogue of SNP which can not release NO and 0.5 mM glucose with 0.5 IU/ml glucose oxidase, respectively, and moreover by combination of GGO with SNP or PFC. Samples of cells were collected at 2-hour intervals during the 12-hour experiment. Viability was detected by double staining with fluorescein diaceate (FDA) and propidium iodide. Oxidoreductase activity was measured by MTT assay. DNA was isolated by CTAB method and its degradation was evaluated on standard agarose gel. Morphology of cells was evaluated by means of fluorescence (FDA staining), bright field and phase contrast microscopy. Acidic compartments were detected by neutral

red (NR) staining. To observe nuclear morphology, cells were fixed and stained by Hoechst 22385.

Results: Only in case of simultaneous action of SNP and GGO cell death appeared (all cells died at the 12th h of the experiment). Contrariwise MTT assay revealed that oxidoreductase activity is decreased in all five treated variants. During the induced cell death nuclei gradually lost rounded shape and chromatin turned to granulated state but only a slight DNA degradation occurred at the end of the experiment. Within cells, the number of vacuoles was reduced and small vesicles appeared in the perinuclear cytoplasmic zone but malformed cells were present in all treated variants. Staining by NR revealed also some acidic compartments.

Conclusion: Treatment of tobacco cells by SNP and GGO induced cell death. We verified that SNP posses NO specific effect in this case, although the ferricyanide moiety influenced the experimental system. Morphology of cells changed and nuclei exhibited features of programmed cell death. Controversially DNA was only slightly degraded.

Acknowledgements

This work was funded by the FRVS No. 180/2004, GACR No. 525/04/P132, IGA MZLU No. 3/2004 and National Research Centre LN00A081.

References

1. Leshem YY: *Nitric Oxide in Plants Occurrence, Function and Use* Dortrecht: Kluwer Academic Publishers; 2000.
2. Delledonne M, Murgia I, Ederle D, Sbicego PF, Biondani A, Polverari A and Lamb C: **Reactive oxygen intermediates modulate nitric oxide signaling in the plant hypersensitive disease-resistance response.** *Plant Physiol Biochem* 2002, **40:**605–610.
3. de Pinto MC, Tommasi F and De Gara L: **Changes in antioxidant systems as part of the signaling pathway responsible for the programmed cell death activated by nitric oxide and reactive oxygen species in tobacco Bright-Yellow 2 cells.** *Plant Physiol* 2002, **130:**698–708.

S35

NO signaling functions in the biotic and abiotic stress responses

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BMC Plant Biology 2005, **5(Suppl 1):**S35

Introduction: Over the past two decades, it has been recognized that nitric oxide (NO) plays an important role in diverse mammalian physiological processes. NO regulates physiological processes by modulating the activity of proteins principally by nitrosylation, a process referring to the binding of NO to a transition metal centre or cysteine residues [1]. An important class of proteins that constitutes key targets of NO is that of the Ca^{2+} channels including plasma membranes as well intracellular Ca^{2+} channels. NO modulates these channels directly by nitrosylation, but also indirectly *via* the second messenger cyclic GMP (cGMP) and/or cyclic ADP ribose (cADPR). Therefore, NO emerges as a key messenger governing the overall control of Ca^{2+} homeostasis [2].

In the late 1990s, NO also became an increasingly popular target for investigation in plants. As in mammals, NO fulfils a broad spectrum of signaling functions in (patho)physiological processes in plants [3]. Here, we summarise studies published in recent years that provide novel insights into the signaling functions of NO produced by plant cells exposed to abiotic stresses and biotic stress (pathogen-derived elicitors). It focuses particularly on the cross-talk operating between NO and Ca^{2+}.

Results: Over the past few years, we have studied the functions of NO in plant cells challenged with elicitors of defense responses. One elicitor has been used primarily: cryptogein, a 10 kDa elicitor produced by the oomycete *Phytophthora cryptogea* [4].

Using the NO sensitive fluorophore 4,5-diaminofluorescein diacetate (DAF-2DA), we reported the real-time imaging of NO production in epidermal tobacco cells treated with cryptogein [5]. After elicitation with the elicitor, the earliest burst of NO was in the chloroplasts, where NO production occurred within 3 minutes. The level of fluorescence increased with time, and after 6 minutes NO was also found along the plasma membrane, in the nucleus and most probably in peroxisomes. To investigate the signaling events that mediate NO production, and to analyse NO signaling activities in the cryptogein transduction pathway, a spectrofluorometric assay using DAF-2DA was developed to follow NO production in tobacco cultured cells. As observed in tobacco epidermal tissue, cryptogein induced a fast and transient NO production in tobacco cell suspensions [6]. This production was completely suppressed in the presence of the NO scavenger cPTIO, and was reduced by 55 to 85% by mammalian nitric oxide synthase inhibitors. By contrast, inhibitors of nitrate reductase inhibitors, a plant NO source, had no effect on cryptogein-induced NO production.

Calcium signals are thought to play an important role in the tobacco cells response to cryptogein [7]. To investigate whether NO was active in this process, the recombinant aequorin technology was used. Aequorin is a photoprotein from *Aequora victoria* which undergoes a conformational change and emits luminescence when occupied by Ca^{2+}. Using transgenic *Nicotiana plumbaginifolia* cell suspensions that constitutively express aequorin in the cytosol, it was shown that cryptogein triggers a biphasic increase of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) resulting from an influx of extracellular Ca^{2+} and Ca^{2+} release from internal stores [7]. When cryptogein-triggered NO production was suppressed by cPTIO or inhibitors of mammalian NOS, the intensity of the first $[Ca^{2+}]_{cyt}$ increase was reduced by almost 50% whereas the second $[Ca^{2+}]_{cyt}$ peak was unaffected. We further provided evidence that NO appears to contribute to the elicitor-induced $[Ca^{2+}]_{cyt}$ elevation by promoting the release of Ca^{2+} from intracellular Ca^{2+} stores into the cytosol [6].

Recently, the role of NO in controlling Ca^{2+} homeostasis was investigated more thoroughly. Its has been shown that NO, released

by the sulphur-free NO donor DEA-NONOate, elicits within minutes a transient influx of extracellular Ca^{2+} and a synchronized increase of $[Ca^{2+}]_{cyt}$ in aequorin-transformed tobacco cells [6]. As predicted from a pharmacological study, the channels responsible for NO-induced $[Ca^{2+}]_{cyt}$ elevation include voltage-dependent Ca^{2+} channels of the plasma membrane and intracellular Ca^{2+} channels sensitive to RYR and IP_3R inhibitors. This observation paralleled the situation encountered in animal cells in which almost all the molecules involved in the control of Ca^{2+} homeostasis seem to be modulated by NO [2]. Recent evidence from our laboratory suggest that NO mediates $[Ca^{2+}]_{cyt}$ through multiple mechanisms including phosphorylation-dependent processes, cADPR and plasma membrane depolarisation.

Our data, along with those from other studies, highlight the crucial role of NO in protecting plants against pathogens by promoting Ca^{2+} mobilization but also defense- and stress-related gene expression and HR [6]. Besides pathogen attack, abiotic stressors, such as drought, salinity and extreme temperature are serious threats to agriculture. In the recent years, a significant amount of work has gone into investigating NO synthesis and functions in plants exposed to abiotic stressors. For example, it was shown both in tobacco leaf peels and tobacco suspension cells that high temperature, osmotic stress, or salinity, generate a rapid and significant surge in NO levels [8]. In contrast, light stress and mechanical injury had no apparent effect on NO production in tobacco and/or tomato. Thus, although NO synthesis can be triggered by several, disparate abiotic stressors, it cannot be considered a universal plant stress response.

Conclusion: Plants express adaptive response to allow them to confer tolerance to environmental stresses and ensure survival. NO function is signal transduction pathways during this response. Although the precise signaling functions of NO are poorly understood, its capacity to modulate Ca^{2+} homeostasis provides an extraordinary and remarkably effective way of conveying information. Little is known about the signaling consequence of the NO/Ca^{2+} crosstalk but it is likely that modulation of the expression of stress-related gene may occur.

References

1. Stamler JS, Lamas S and Fang FC: **Nitrosylation: the prototypic redox-based signalling mechanism.** *Cell* 2001, **106:**675–683.
2. Clementi E: **Role of nitric oxide and its intracellular signalling pathways in the control of Ca^{2+} homeostasis.** *Biochem Pharmacol* 1998, **55:**713–718.
3. Wendehenne D, Durner J and Klessig DF: **Nitric oxide: a new player in plant signalling and defence responses.** *Curr Opin Plant Biol* 2004, **7:**449–455.
4. Wendehenne D, Lamotte O, Frachisse JM, Barbier-Brygoo H and Pugin A: **Nitrate efflux is an essential component of the cryptogein signaling pathway leading to defense responses and hypersensitive cell death in tobacco.** *Plant Cell* 2002, **14:**1937–1951.
5. Foissner I, Wendehenne D, Langebartels C and Durner J: ***In vivo* imaging of an elicitor-induced nitric oxide burst in tobacco.** *Plant J* 2000, **23:**817–824.
6. Lamotte O, Gould K, Lecourieux D, Sequeira-Legrand A, Lebrun-Garcia A, Durner J, Pugin A and Wendehenne D: **Analysis of nitric oxide signaling functions in tobacco cells challenged by the elicitor cryptogein.** *Plant Physiol* 2004, **135:**516–529.
7. Lecourieux D, Mazars C, Pauly N, Ranjeva R and Pugin A: **Analysis and effects of cytosolic free calcium increases in response to elicitors in *Nicotiana plumbaginifolia* cells.** *Plant Cell* 2002, **14:**2627–2641.
8. Gould KS, Lamotte O, Klinguer A, Pugin A and Wendehenne D: **Nitric oxide production in tobacco leaf cells: a generalized stress response?.** *Plant Cell Environ* 2003, **26:**1851–1862.

S36

Does tubulin phosphorylation correlate with cell death in plant cells?

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BMC Plant Biology 2005, **5(Suppl 1):**S36

Background: Microtubules are necessary for a wide spectrum of cellular functions, which include cell division, intracellular transport, organelle positioning and generating of cell polarity. The major component of microtubules is tubulin heterodimeric protein which is consist of two subunits: α- and β-tubulin. Both tubulin subunits can be extensively altered by post-translational modifications, including detyrosination/tyrosination, acetylation/deacetylation, polyglutamylation, polyglycylation, and phosphorylation. As for the different tubulin isotypes, the functionality of the post-translational modifications is still a matter of debate. Although, it is known that some of them are associated with stable/dynamic populations of microtubules, while others seem to influence the binding of motor proteins [1]. One of post-translational modifications, tubulin phosphorylation, is not a widely observed and its precise function is unknown both in animal and plant cells.

It was shown recently that animal tubulin can be phosphorylated by different systems of cyclic nucleotide-dependent (cAMP- and cGMP-dependent) protein kinases, Ca^{2+}-dependent protein kinases (including Ca^{2+}-calmodulin-dependent and Ca^{2+}-dependent, phospholipid-stimulated types of enzymes), casein kinases and tyrosine kinases, too [2, 3]. The combined data demonstrate that plant tubulin can also undergo extensive phosphorylation by different types of protein kinases and that the phosphorylation on serine\threonine as well as at tyrosine residues can participate in the generation of high level of polymorphism of plant tubulin [4]. It is interesting to establish a functional role of this tubulin modification as phosphorylation is a universal post-translational modification which is typical for most of the proteins. The effects of different activators and inhibitors of protein kinases on microtubule dynamics and cell cycle progression in plan cells are present in this report.

Materials and methods: Two plant lines, *Arabidopsis thaliana* [5] and tobacco BY-2 cell culture [6] (kindly handed over by Prof. J.-P. Verbelen, University of Antwerp, Belgium) both expressing GFP-tubulin as well as *A. thaliana* and *Nicotiana tabacum* wild types were used in this research. GFP-labeled microtubules in *A. thaliana* and BY-2 cells were analyzed by confocal laser scanning microscopy.

The root tips of 3-day old *Allium cepa* seedlings were also used in this study. The primary mouse monoclonal antibodies TU-01 (against α-tubulin) and TU-06 (against β-tubulin) (kindly provided by Drs. V. Viklicky and P. Draber, Institute of

Molecular Genetics, Prague, Czech Republic) were used for visualisation of microtubules in onion meristematic root tip cells by immunofluorescence microscopy. FITC-conjugated anti -mouse antibody (Sigma, USA) was used as a secondary one. The fixation and staining of microtubules by antibodies were performed as described by us early [7].

As regulators of protein kinases, dibutyryl-cAMP (Serva, Germany) in combination with ATP, polymyxin B (Serva, Germany), trifluoperazine (Serva, Germany) and okadaic acid (Sigma, USA) were used.

Results: For more detailed analysis of the functional role of tubulin phosphorylation in plant cells several specific inhibitors and activators of different types of protein kinases were used in our research. Dibutyryl-cAMP (10 μM in combination with 100 μM ATP) as an activator of cAMP-dependent phosphorylation, polymyxin B (5 mM) as an inhibitors of the protein kinase C, trifluoperazine (5 mM), as an inhibitor of the Ca^{2+}-calmodulin-dependent protein kinase, and okadaic acid (inhibitor of protein phosphatase type 2A, PP2A), in concentration 1–30 nM, were investigated with regard to their ability to affect microtubule dynamics and to induce structural changes of microtubules. The root tips of seedlings were treated with each of these compounds. The effects of these regulators of protein kinases on the structural reorganisation of interphase and mitotic microtubules were studied after exposure of plant material in the presence of activator or inhibitor during 6, 12 and 24 h.

Immunofluorescence analysis of microtubules showed that treatment by cAMP causes the disruption of both interphase and mitotic microtubules and accumulation of depolymerised tubulin around the nuclei in the cells. The treatment of onion cells by trifluoperazine caused the reorganization of microtubules and change of their spatial organization from a transverse to a longitudinal orientation and formation of thick longitudinal arrays. The treatment of *A. cepa* cells with polymyxin B caused the same effects on microtubular organisation as trifluoperazine.

Confocal laser scanning and light microscopy of *A. thaliana* and *N. tabacum* cells revealed that okadaic acid arrested cell growth, alter cell morphology, and affected the organization of microtubules.

Conclusion: It was reviewed by us that plant tubulin can undergo extensive phosphorylation by different types of protein kinases, and that tubulin phosphorylation participates in regulation of the plant cell cycle [4]. Many studies shown that different protein phosphatase inhibitors effect microtubules in animal and plant cells. For instance, it was shown that the treatment of *Tradescantia* stamen hair cells with okadaic acid and other protein phosphatase inhibitors caused changes of the metaphase transit times and the pattern of sister chromatid separation [8]. The treatment of *Arabidopsis* shoots with inhibitors of serine/threonine protein phosphatases (okadaic acid or calyculin A) provoked the destruction of root morphology, that can be explained by the influence of these compounds on cortical microtubules function [9]. The same authors later proved that phosphatase inhibitors as well as protein kinase inhibitors destroy not only root morphology but that cortical microtubules also become disorganized after exposure to some types of inhibitors [10]. In particular, these effects were characteristic of protein phosphatases such as calyculin A and cantaridin. The protein kinase inhibitor staurosporine also had similar effect in plant cells [11, 12, 13]. The disruption of microtubules was found recently after calyculin A and okadaic acid treatment in *Lilium* [14]. Thus, literature indicates that phosphorylation and dephosphorylation represent a part of the molecular mechanism responsible for both the organization of the cortical microtubular networks and of mitotic function.

Studies on animal cells clearly demonstrated that okadaic acid and other protein phosphatase inhibitors induce mitotic arrest [15, 16], premature chromosome condensation [17, 18], microtubule disassembly [18, 19], DNA fragmentation [20, 21] and apoptosis [16, 17, 20, 21].

Summarizing our data obtained we can conclude that the changes in the spatial organisation of microtubules after treatment by cAMP and the protein kinase inhibitors lead to disturbances of cell cycle progression and it is most likely to launch of the cell death program in plant cells.

Acknowledgements

This work was supported partly by INTAS grant N 03-51-6459.

References

1. Westermann S and Weber K: **Post-translational modifications regulate microtubule function.** *Nature Rev Mol Cell Biol* 2003, **4:**938–947.
2. Luduena RF: **Multiple forms of tubulin: different gene products and covalent modifications.** *Int Rev Cytol* 1998, **178:**207–275.
3. MacRae TH: **Tubulin post-translational modifications. Enzymes and their mechanisms of action.** *Eur J Biochem* 1997, **244:**265–278.
4. Blume YaB, Yemets AI and Lloyd CW: **Plant tubulin phosphorylation and its role in cell cycle progression.** *NATO Series* .
5. Ueda K, Matsuyama T and Hashimoto T: **Visualization of microtubules in living cells of transgenic *Arabidopsis thaliana*.** *Protoplasma* 1999, **206:**201–206.
6. Kumagai F, Yoneda A, Tomida T, Sano T, Nagata T and Hasezawa S: **Fate of nascent microtubules organized at the M/G1 interface, as visualized by synchronized tobacco BY-2 cells stably expressing GFP-tubulin: time-sequence observations of the reorganization of cortical microtubules in living plant cells.** *Plant Cell Physiol* 2001, **42:**723–32.
7. Yemets AI, Kundel'chuk OP, Smertenko AP, Solodushko VA, Rudas VA, Gleba YY and Blume YB: **Transfer of amiprophosmethyl-resistance from a *Nicotiana plumbaginifolia* mutant by somatic hybridisation.** *Theor Appl Genet* 2000, **100:**847–857.
8. Wolniak SM and Larsen PM: **Changes in the metaphase transit times and the pattern of sister chromatid separation in stamen hair cells of *Tradescantia* after treatment with protein phosphatase inhibitors.** *J Cell Sci* 1992, **102:**691–6715.
9. Smith RD, Wilson JE, Walker JC and Baskin TI: **Protein phosphatase inhibitors block root hair development and alter cell shape in *Arabidopsis* roots.** *Planta* 1994, **194:**516–524.
10. Baskin TI and Wilson JE: **Inhibitors of protein kinases and phosphatases alter root morphology and disorganize cortical microtubules.** *Plant Physiol* 1997, **113:**493–502.
11. Hasezawa S and Nagata T: **Okadaic acid as a probe to analyse the cell cycle progression in plant cells.** *Bot Acta* 1992, **105:**63–69.

12. Mizuno K: **Inhibition of gibberelin-induced elongation, reorientation of cortical microtubules and change of isoform of tubulin in epicotyl segments of azuki bean by protein kinase inhibitors.** *Plant Cell Physiol* 1994, **35:**1149–1157.
13. Zhang K, Tsukitani Y and John PCL: **Mitotic arrest in tobacco caused by the phosphoprotein phosphatase inhibitor okadaic acid.** *Plant Cell Physiol* 1992, **33:**677–688.
14. Foissner I, Grolig F and Obermeyer G: **Reversible protein phosphorylation regulates the dynamic organization of the pollen tube cytoskeleton: effects of calyculin A and okadaic acid.** *Protoplasma* 2002, **220:**1–5.
15. Wera S and Hemmings BA: **Serine/threonine protein phosphatases.** *Biochem J* 1995, **311:**17–29.
16. Lerga A, Richard C, Delgado MD, Caneles M, Frade P, Cuadrado MA and Leon J: **Apoptosis and mitotic arrest are two independent effects of the protein phosphatase inhibitor okadaic acid in K562 leukemia cells.** *Biochem Biophys Res Comun* 1999, **260:**256–264.
17. Ishida Y, Furukawa Y, Decaprio JA, Saito M and Griffin JD: **Treatment of myeloid leukemic cells with phosphatase inhibitor okadaic acid induces cell cycle arrest at either G1/S or G2/M depending on dose.** *J Cell Physiol* 1992, **150:**484–492.
18. Sun Q-Y, Wu G-M, Lai L, Bonk A, Cabot R, Park K-W, Day BN, Prather RS and Schatten H: **Regulation of mitogen-activated protein kinase phosphorylation, microtubule organization, chromatin behavior, and cell cycle progression by protein phosphatases during pig oocyte maturation and fertilization in vitro.** *Biol Reprod* 2002, **66:**580–588.
19. Zhang L, da Costa SR, Yarber FA, Runnegar M and Hamm-Alvarez SF: **Protein phosphatase inhibitors alter cellular microtubules and reduce carbachol-dependent protein secretion in lacrimal acini.** *Curr Eye Res* 2000, **20:**373–383.
20. Inomata M, Saijo N, Kawashima K, Kaneko A, Fujiwara Y, Kunikana H and Tanaka Y: **Induction of apoptosis in cultured retinoblastoma cells by the protein phosphatase inhibitor, okadaic acid.** *J Cancer Res Clin Oncol* 1995, **121:**729–738.
21. Nuydens R, Dispersyn G, van den Keiboom G, de Jong M, Connors R, Ramaekers F, Borgers M and Geerts H: **Bcl-2 protects against apoptosis-related microtubule alterations in neuronal cells.** *Apoptosis* 2000, **5:**43–51.

S37

Nocodazole provokes an apoptosis in isopropyl-*N*-phenyl carbamate resistant and sensitive *Nicotiana* lines but in two different ways

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BMC Plant Biology 2005, **5(Suppl 1):**S37

Background: Microtubules are highly dynamic assemblies of the protein tubulin. Their dynamic behaviour is crucial to mitosis, the process of chromosomal division to form new cells. Microtubule dynamics are highly regulated during the cell cycle by endogenous cellular regulators. In addition, many antimitotic drugs and natural compounds alter the polymerisation dynamics of microtubules, blocking mitosis, and consequently, inducing cell death by apoptosis [1]. The drugs bind to diverse sites on tubulin and at different positions within the microtubule, and they have diverse effects on microtubule dynamics. However, by their common mechanism of suppression microtubule dynamics, they all block mitosis at the metaphase/anaphase transition, and induce cell death. After treatment with different anti-tubulin drugs, it was also demonstrated that apoptosis occurs either in the interphase or in mitosis depending on the cell type and experimental conditions [2, 3].

The relationship between cell cycle disturbance due to the use of these agents and apoptosis is poorly understood. Insight into the binding and mode of action of most of antimitotic drugs have been obtained by the identification and mapping of mutation in appropriate resistant lines. Unfortunately, up to now there is no strong evidence about mode of action *N*-phenyl carbamates, effective anti-mitotic herbicides. Although, recently it was shown that phenylcarbamates and benzimidazoles, can compete for the site(s) of binding on β-tubulin. Benzimidazoles interact very specifically with different eukaryotic β-tubulin and resistance to these anti-microtubule drugs may be caused by point mutations in β-tubulin which replace Glu-198 with either Ala, Asp, Gln, Lys, Val, or Gly, or which replace Phe-200 with Tyr [4]. As it was found amino acid substitutions in position 198 of fungal β-tubulin, lead to negative cross-resistance to phenylcarbamates, but changes Phe from Leu-250, Val from Ala-165, and Ala from Thr-237 are responsible for phenylcarbamate cross-ressistance [5]. To clarify the sensitive/resistance of isopropyl-*N*-phenylcarbamate (IPC) resistant *N. sylvestris* mutants [6] to benzimidazole the influence of different concentration of nocodazole and its diverse effects on microtubule dynamics and cell cycle progression were studied. Two different apoptotic responses and patterns in control and mutant lines were found after nocodazole treatments.

Materials and methods: Nocodazole (Sigma) was dissolved in dimethylsulfoxide (DMSO) and stored at –20°C. The final DMSO concentration during nocodazole treatments did not exceed 0.5%. Only 0.5% DMSO was added to control samples. The root cells of control and IPC-resistant mutant *N. sylvestris* lines were exposured with nocodazole at various concentrations (0.01, 0.1, 1, 10, 100, and 500 MkM) for 24 h, and then fixed in an ethanol/acetic acid mixture (3:1) for 12 h. For chromosome counting and classification of mitotic figures, slides, were stained with acetoorcein (1% solution in 45% acetic acid) for 24 h and examined. Cell were counted and analysed at magnification of 1000X on Carl Zeiss light microscope.

Results: In both control and IPC-resistant *N. sylvestris* lines various nocodazole concentration treatments led not only to increase the mitotic indexes from about 7% to ~12–13%, but also resulted in an appearance of mitotic figure disturbances during different phases of mitotic division (see Table 1 and Table 2). No any disturbances were found in prophases of IPC-resistant plants after drug effects, whereas nocodazole in concentration from 10 to 500 MkM revealed them in control plant cells on dose-depended manner (Table 1). It was found that metaphases of both lines were more sensitive to nocodazole treatments, but nevertheless the per cent of

Table 1 (abstract S37) Results of different nocodazole concentration effects on cell cycle progression of control *N. sylvestris* line

NZ, MM	MI, %	Σ analysed cells, number	Σ dividing cells, number	Mitotic phases							
				Prophase, % in all /disturbances		Metaphase, % in all /disturbances		Anaphase, % in all /disturbances		Telophase, % in all /disturbances	
500	11.58	7022	813	32.84	18.33	35.19	31.86	18.77	16.48	13.16	10.95
100	10.66	7358	784	44.39	4.41	30.31	27.04	15.60	12.88	9.73	7.78
50	9.98	7014	699	51.22	2.88	28.31	24.61	12.01	9.16	8.44	6.01
10	9.72	6129	596	57.72	0.40	25.50	17.11	9.73	5.54	7.08	3.36
1	8.93	6174	551	60.62	0	23.05	14.70	9.26	4.54	7.01	2.90
0.1	7.68	7412	569	67.66	0	17.58	7.73	8.26	1.58	6.51	0.88
0.01	7.65	7062	540	74.23	0	15.19	2.04	4.63	0	5.93	0
0	7.54	7135	538	60.04	0	20.22	0	8.36	0	11.35	0

NZ = nocodazole, MI = mitotic index, % of disturbances in each mitotic phase was calculated as % from total number of dividing cells.

Table 2 (abstract S37) Results of different nocodazole concentration effects on cell cycle progression of IPC-resistant *N. sylvestris* line

NZ, MM	MI, %	Σ analysed cells, number	Σ dividing cells, number	Mitotic phases							
				Prophase, % in all / disturbances		Metaphase, % in all / disturbances		Anaphase, % in all / disturbances		Telophase, % In all / disturbances	
500	13.54	7045	954	56.18	0	21.11	19.49	12.17	10.79	10.57	8.49
100	12.03	7748	932	66.42	0	15.67	14.16	9.72	8.15	8.18	6.22
50	10.73	7036	755	72.05	0	14.82	8.21	7.39	3.05	5.66	2.65
10	10.27	6198	636	76.89	0	12.26	6.13	6.13	2.04	4.72	1.89
1	9.98	6156	614	82.89	0	9.33	3.91	4.65	1.47	3.22	0.49
0.1	8.52	7015	597	85.93	0	8.67	3.18	2.59	0.34	2.77	0.34
0.01	7.23	7098	513	89.28	0	7.53	0	1.22	0	1.99	0
0	6.67	7159	478	74.48	0	17.51	0	4.81	0	3.06	0

NZ = nocodazole, MI = mitotic index, % of disturbances in each mitotic phase was calculated as % from total number of dividing cells.

disturbances in metaphases, anaphases and telophases was about in two-three times more in control line as compare to mutant one (see Table 1 and 2).

It was found also that different nocodazole concentrations rapidly induced apoptotic processes in both control and mutant lines, but cell responses after nocodazole treatments were completely different. Even low concentration of nocodazole (0.1–10 MkM) induced nuclear DNA damage: chromatin recondensation and DNA fragmentation in prophases, metaphases and anaphases of control plant cells (see Figure 1). Whereas high level of nocodazole (100–500 MkM) induced total DNA fragmentation on all stages of cell cycle including interphase.

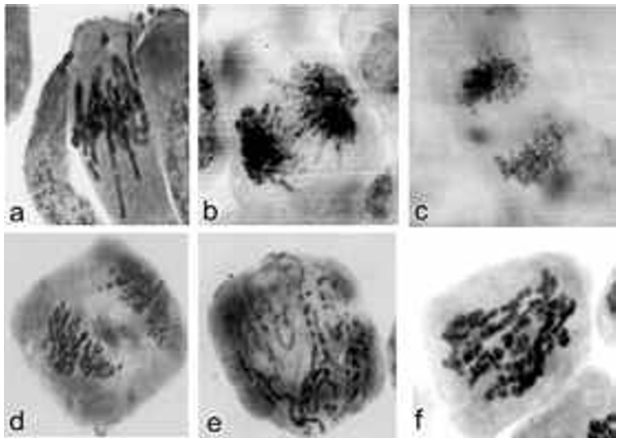
At concentration of 0.1 MkM nocodazole effected significantly metaphases only in IPC-resistant plant cells. It was found that in the cells of mutant lines treatment of low nocodazole level (0.1–1 MkM) resulted in chromosome delay during chromosome segregation in metaphase, 10 MkM nocodazole treatment induced an appearance of C-mitosis, chromosome delay and chromosome-bridges formation in different phases of mitosis (Figure 2).

High levels of nocodazole (100–500 MkM) resulted in dramatic alterations in the cells – C-mitosis, aberrant spindle formation with consequent more frequent anomalous situations (for example, multipolar mitoses, and as a result of this, an appearance of star anaphases). No any DNA fragmentation was observed in IPC-resistant lines after any concentration of nocodazole treatment.

Conclusion: It is well known that treatment of cells with nocodazole and other microtubule-interfering agents evokes the activation of stress response pathways, cell cycle arrest, and the induction of apoptosis. Early, nocodazole was frequently used to examine the role of microtubules in vesicle transport [7], to induction of polyploidy [8], aneuploidy [9], and certainly to study an apoptosis in different cells [8, 9, 10, 11, 12, 13]. Very often nocodazole effects result in DNA fragmentation, one hallmark of apoptosis. In our data nocodazole treatments revealed DNA fragmentation in control, sensitive to IPC, *N. sylvestris* line only. It was not found any DNA fragmentation in mutant plan cells after nocodazole exposure. Nevertheless, cell death was observed in both lines.

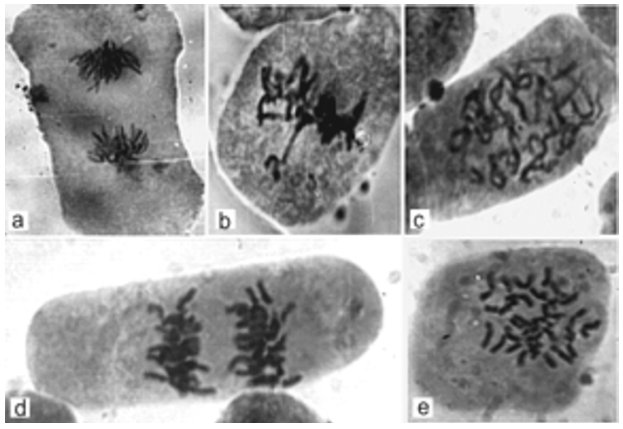
We established that nocodazole increased the mitotic index in both lines but it also enhanced an apoptotic response. These results are coincided with data obtained early on animal cells [10]. We also found that IPC-resistant *N. sylvestris* mutant cells possessed less sensitivity to nocodazole treatments that control

Figure 1 (abstract S37)



Mitotic figures in sensitive *N. sylvestris* lines after treatment of nocodazole in various concentrations: (**a**) 0.1 MkM nocodazole treatment; (**b-c**) 1 MkM nocodazole treatment; (**d-f**) 10 MkM nocodazole treatment. Bar: 1 cm = 1000 mm.

Figure 2 (abstract S37)



Mitotic figures in IPC-resistant *N. sylvestris* lines after treatment of nocodazole in various concentrations: (**a-b**) 1 MkM nocodazole treatment; (**c-d**) 10 MkM nocodazole treatment; (**e**) 100 MkM nocodazole treatment. Bar: 1 cm = 1000 mm.

N. sylvestris plant since it was established that the per cents of disturbances in metaphases, anaphases and telophases were about in two-three times more in *N. sylvestris* control line as compare with mutant one. Microtubules in prophase of mutant plant cells were resistant to all used nocodazole concentrations. As it was shown there were no disturbances on this stage of mitosis in IPC-resistant plants. However, all revealed disturbances in metaphase, anaphase and telophase, in mutant testify about cell suicide program after this drug effect.

Thus, we can summarize that IPC-resistant plant cells are more resistant to nocodazole than that of control line. Nocodazole in concentration from 0.1 to 500 MkM evokes clear apoptosis in control *N. sylvestris* plant cells characterizing in mitotic arrests and DNA fragmentation. It provokes also cell death process in IPC-resistant cells, but on another distinct way of cell degradation. It is possible to conclude that the same nocodazole treatments could induce, in two different plant lines, cytostatic effects followed by probably two different patterns of plant death. Recently, two different patterns of cell death were also found on two different cell animal lines after same antimicrotubular drug effects [3]. It has already been reported early that nocodazole possesses pleiotropic effects that are independent of microtubules [7], or probably different apoptotic responses depend from cell types as it was indicated by us above.

It is possible to presume that the higher apoptotic rate in control plant cells after nocodazole treatments may be also a consequence of absence mutant microtubule protein(s) or mutant microtubule associated protein(s) which confer(s) resistance to IPC in *N. sylvestris* mutant lines. Experiments on nocodazole effects on cells of model plant lines are continuing now.

References

1. Mollinedo F and Gajate C: **Microtubules, microtubule-interfering agents and apoptosis.** *Apoptosis* 2003, **8:**413–450.
2. Tsuchida T, Yoshimura K, Shirayama Y and Kawamoto K: **Colcemid-induced apoptosis of cultured human glioma: electron microscopic and confocal laser microscopic observation of cells sorted in different phases of cell cycle.** *Cytometry* 1998, **31:**295–299.
3. Sciola L, Spano A, Monaco G, Bottone MG and Barni S: **Different apoptotic responses and patterns in adhering and floating neoplastic cell cultures: effects of microtubule antagonists.** *Histochem Cell Biol* 2003, **119:**77–90.
4. Davidse LC and Ishi H: **Biochemical and molecular aspects of the mechanisms of action of benzimidazole, *N*-phenylcarbamates and *N*-phenylformamidoximes and the mechanisms of resistance to these compounds in fungi.** *Antifungal Agents – Discovery and Mode of Action* BIOS Scientific Publishers Ltd: Dixon GK, Copping LG, Hollomon DW 1995, 305–321.
5. Fujimura M, Kamakura T, Inoue H and Yamaguchi I: **Amino-acid alterations in the beta-tubulin gene of *Neurospora crassa* that confer resistance to carbendazim and diethofencarb.** *Curr Genet* 1994, **25:**418–22.
6. Yemets A, Stelmakh O, Kundelchuk O and Blume YaB: **Obtaining and analysis of isopropyl-*N*-phenyl carbamate resistant lines of *Nicotiana* species.** *Cell Biol Int* 2003, **27:**307–310.
7. Molero JC, Whitehead JP, Meerloo T and James DE: **Nocodazole inhibits insulin-stimulated glucose transport in 3T3-L1 adipocytes via a microtubule-independent mechanism.** *J Biol Chem* 2001, **276:**43829–43835.
8. Verdoodt B, Decordier I, Geleyns K, Cunha M, Cundari E and Kirsch-Volders M: **Induction of polyploidy and apoptosis after exposure to high concentrations of the spindle poison nocodazole.** *Mutagenesis* 1999, **14:**513–520.
9. Decordier I, Dillen L, Cundari E and Kirsch-Volders M: **Elimination of micronucleated cells by apoptosis after treatment with inhibitors of microtubule.** *Mutagenesis* 2002, **17:**337–344.
10. Bumbasirevic V, Skaro-Milic A, Mircic A and Djuricic B: **Apoptosis induced by microtubule disrupting drugs in normal murine thymocytes in vitro.** *Scanning Microsc* 1995, **9:**509–516.

11. Ikegami R, Zhang J, Rivera-Bennetts AK and Yager TD: **Activation of the metaphase checkpoint and an apoptosis programme in the early zebrafish embryo, by treatment with the spindle-destabilisin agent nocodazole.** *Zygote* 1997, **5:**329–350.
12. Kook S, Shim SR, Kim JI, Ahnn JH, Jung YK, Paik SG and Song WK: **Degradation of focal adhesion proteins during nocodazole-induced apoptosis in rat-1 cells.** *Cell Biochem Funct* 2000, **18:**1–7.
13. Zhang H, Shi X, Zhang Q-J, Hampong M, Paddon H, Wahyuningsih D and Pelech S: **Nocodazole-induced p53-dependent c-Jun N-terminal kinase activation reduces apoptosis in human colon carcinoma HCT116 cells.** *J Biol Chem* 2002, **227:**43648–43658.

S38

Early PCD events in male gametophyte development of isopropyl-*N*-phenyl carbamate resistant *Nicotiana sylvestris* mutant lines

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BMC Plant Biology 2005, **5(Suppl 1):**S38

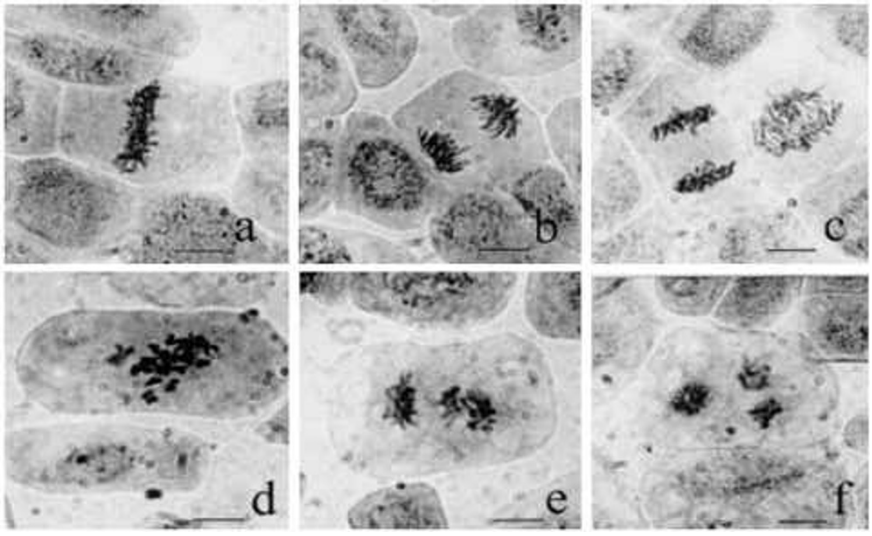
Background: Microtubules provide structural support for cells and play a key role in mitosis and meiosis. They also play a key role in plant cell differentiation, new tissue and organ formation through a coordinated program of symmetric or asymmetric cell division. It is well known that microtubule-damaging agents suppress microtubule dynamics, leading to disruption of the mitotic spindle in dividing cells, cell cycle arrest at M-phase, and the late apoptosis [1]. A better understanding of the processes coupling microtubule damage to the onset of apoptosis will help to find out in process of higher plants development. Recently, the mutant *Nicotiana* lines with high resistance to antimitotic herbicide isopropyl-*N*-phenyl carbamate (IPC) were produced [2, 3]. Detailed analysis of IPC-resistant *Nicotiana sylvestris* plants revealed some abnormalities in microsporogenesis leading to partial pollen sterility. Here we report that these mutant *N. sylvestris* lines conferring resistance to IPC demonstrate clearly programmed cell death (PCD) events in male gamethophyte formation and development.

Materials and methods: IPC-resistant and control (sensitive) *N. sylvestris* plants grown in *in vitro* conditions and greenhouse were used in this study. Sterile plants of both lines were placed from *in vitro* conditions to soil for adaptation, and then they were grown in greenhouse.

For cytological analysis the roots of control and mutant *N. sylvestris* lines were treated with 30 mkM IPC for 24 h, fixed in an ethanol/acetic acid mixture (3:1) for 12 h, stained with acetoorcein (1% solution in 45% acetic acid) for 24 h and examined. Bud seeds and anthers were fixed accordingly protocols [4]. After washing the samples were kept in 70% ethanol. The samples were underwent an alkali and enzyme treatment before staining with acetoorcein.

Results: The resistance to IPC in mutant *N. sylvestris* lines were confirmed by us by series of tests and morphocytochemical analyses. For instance, the morphocytochemical analysis of control and mutant lines revealed 6–8 times more resistance to IPC of mutant line as compared with control one. It was found also that the increasing doses of IPC lead to dramatic changes in nucleus and microtubule network in sensitive plants. In control plants tripolar anaphases and telophases were found after treatment with 30 MkM IPC. IPC-treatment resulted also in high number of metaphase+anaphase (from 32.48% in control cells

Figure 1 (abstract S38)



Mitotic figures in resistant (a-c) and sensitive (d-f) N. sylvestris lines after 30 MkM treatment. Bar = 1000 mm.

without treatment to 80.07% after treatment) and accumulation of multinuclear cells and lobbed nuclei in treated control cells (see Figure 1d–f).

Threshold level of IPC induced an apoptotic process, characterised by cell cycle arrest, hypercondensation of chromatin, an appearance of lobbed nuclei; abnormal, multipolar mitotic spindles, post-mitotic micronuclei formation due to nuclei fragmentation in the root cells of sensitive lines. However, root meristematic cells from resistant plants revealed no disturbances in cell cycle progression after treatment with 30 MkM IPC (see Figure 1a–c).

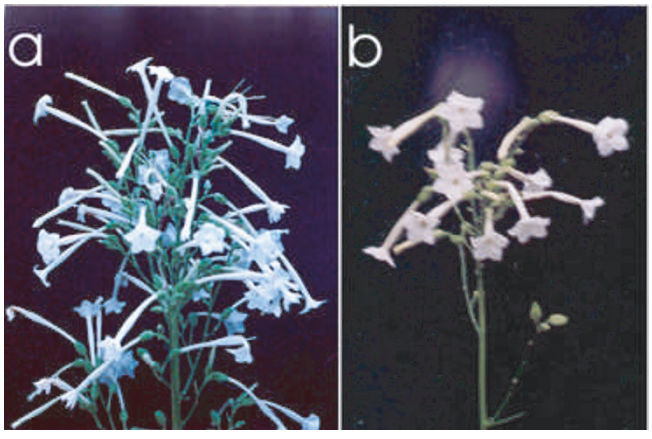
Further investigations shown that control and mutant *N. sylvestris* plants possessed some morphological differences (see Figure 2). Mutant plants characterised in certain depression resulted in reduction of sizes of vegetative and generative organs, and of plant bodies on the whole. There were slight differences in the appearance of flowers (see Figure 3a) and more visible differences in the length of stamens and pistils between control and mutant lines (see Figures 3b and 3c).

More detailed analysis of reproductive organs development of IPC-resistant mutants revealed that microspores in anthers are subjected to degenerative process, leading to pollen sterility. It was found also the chromatin agglutination in nuclei of most part of microsporocytes.

During study the microsporogenesis in IPC-mutant plants it was revealed the formation of microspores of different sizes (gigantic, medium and dwarfic: 15.05 ± 0.06 mkm, 7.50 ± 0.23 mkm and 5.15 ± 0.13 mkm in size, respectively) and in different numbers (dyads, triads and polyads). In the same time normal tetrads produced in control *N. sylvestris* plants (data not shown). Variation in sizes and numbers indicates on disturbances in chromosome separation in I and II meiotic divisions, loss the second division in one (in the case of triads) and in both dyads, the formation of multipolar spindles (in the case of polyads) in mutants.

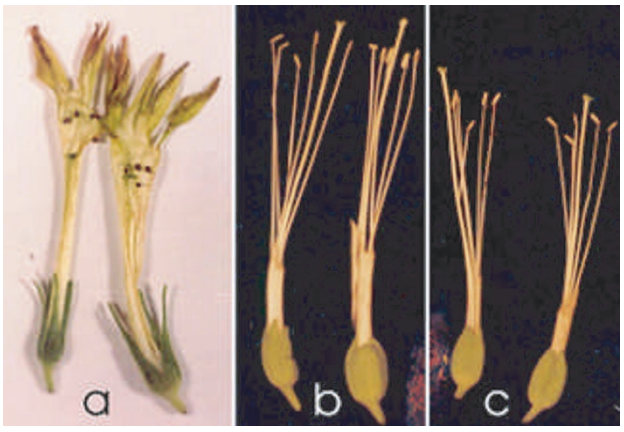
It was established that pollen grains of control plants possessed the high level of fertility (97.12 ± 0.33%); they were homogenous and synchronous in development. Whereas, genetically non-balanced microscopes of mutant lines after tetrad dissipation were unable to

Figure 2 (abstract S38)



The view of flowering parts of *N. sylvestris* control (**a**) and 30 MkM IPC-resistant (**b**) lines.

Figure 3 (abstract S38)



(a) An appearance of flowers of *N. sylvestris* control (left) and mutant (right) lines; Flower generative organs of *N. sylvestris* control **(b)** and mutant **(c)** lines.

further development. They did not synthesise the fats and new cytoplasm and underwent typical cell death. Only 14.73 ± 1.09% formed microspores got down later to division with vegetative and generative cells formation.

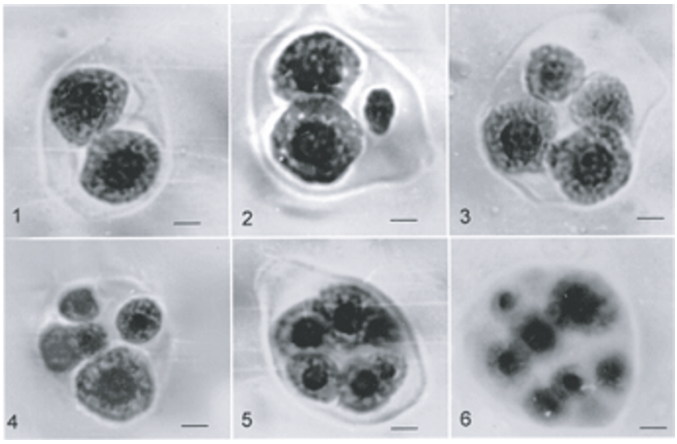
As a result, the common number of pollen was reduced in anthers of mutant plants. About 2.88 ± 0.33% of bicellular pollen grains were hydrated and stopped in development. Consequently, the mature pollen of mutant plants was morphologically and functionally non-homogenous. Its fertility in different flowers was from 7.63 to 27.93%. Sterile pollen grains were small, weakly stained or non-stained after incubation with dye (see Figure 5b). They also had reduced contents of cytoplasm as compared with control plants.

The results of female gametophyte study in mutant and control *N. sylvestris* plants demonstrated that ovary development after pollination was lower in 1.5–2 times in mutant plants as compare with control ones (see Figure 6). The delay in growth and development was connected with the high number of non-fertilized bud seeds (see Figure 7) in mutant plants. We found strong correlation between fertility level of pollen and a number of fertilized bud seeds in these plants.

Analysis of fertilized bud seeds of *N. sylvestris* mutants shown that bud seeds were different in the sizes that indicates on their non-homogeneous and asynchronous development as compare with control plants. It was found that a speed of embryos development in mutant plants was lower than that in control lines, and sometimes an embryo development stopped on early globular stage. Therefore, the seed production in mutant plants was very low due to both high pollen sterility and decreased viability of bud seeds development.

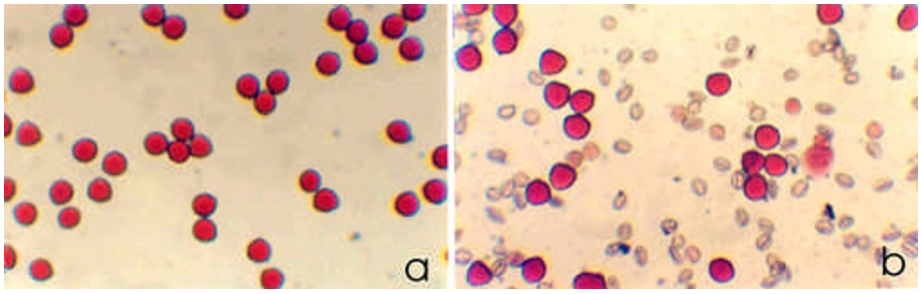
Conclusion: Programmed cell death has been observed to occur during many stages of plant reproduction [5] as PCD is essential for development and survival of plants. But there are still a lot of open questions about genes and their products which might function as signals to evoke a cell suicide program, in particular, during male gametophyte development. Comprehensive reviews about the mutants known to affect meiosis [6] and about the genes controlled of male gametophyte

Figure 4 (abstract S38)



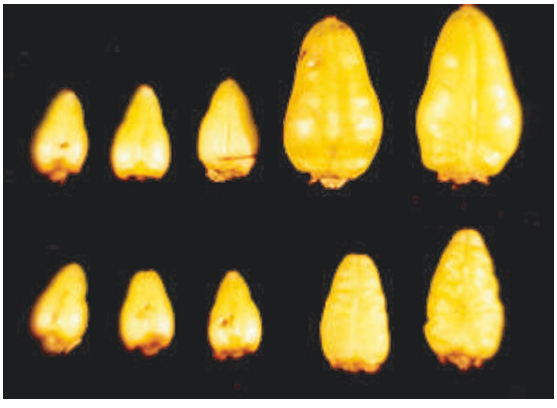
Disturbances on the stage of late tetrad in *N. sylvestris* mutant plant anthers: 1 — dyad, 2 — triad, 3 — atypical tetrad, 4 — pentad, 5 — hexad, 6 — polyad. Bar = 0.005 mm.

Figure 5 (abstract S38)



(a) Mature pollen grains from *N. sylvestris* control **(a)** and mutant **(b)** lines. Fertile pollen grains are stained, non-fertile are not stained by acetoorcein.

Figure 6 (abstract S38)



Ovaries of *N. sylvestris* control (at the top of the picture) and mutant (below on the picture) lines straight away after pollination, and after one, two, three and four weeks of development.

development [7] help to understand and clarify certain moments in this issue, but not completely. We suggest also that cytoskeleton proteins, in particular, different isoforms of alpha-, beta- and gamma-tubulins as well as other microtubule organising centre (MTOC) proteins may play a crucial role in many processes of microsporogenesis.

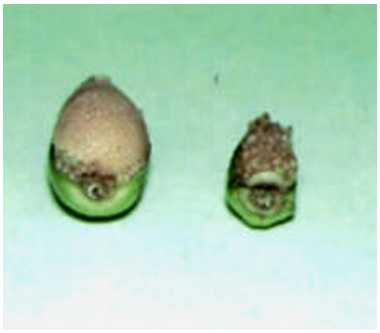
Summarising obtained results we can consider that all established abnormalities in male reproductive organ development leading to PCD in pollen production can be arise from a mutation of the microtubule protein(s) in IPC-resistant *N. sylvestris* lines. We hypothesize also that the mutant protein (s) may represent(s) a key biological trigger in the expression of physiological death at the cellular level in these plants.

The analysis of IPC-resistant *N. sylvestris* plants is continuing to determine the role of certain mutant cytoskeletal gene(s) which is/are express(es) in male gametophyte and lead to PCD during pollen production for better understanding the male reproductive organ development.

References

1. Mollinedo F and Gajate C: **Microtubules, microtubule-interfering agents and apoptosis.** *Apoptosis* 2003, **8:**413–450.

Figure 7 (abstract S38)



Three-weeks old ovaries in section of *N. sylvestris* control (left) and mutant (right) plants after pollination.

2. Yemets AI, Mileyko AA and Blume YaB: **Obtaining the mutants of *Nicotiana plumbaginifolia* L. resistant to herbicide isopropyl-*N*-phenylcarbamate.** *Proc Natl Acad Sci Ukraine* 2000, **10:**182–187.
3. Yemets A, Stelmakh O, Kundelchuk O and Blume YaB: **Obtaining and analysis of isopropyl-*N*-phenyl carbamate resistant lines of *Nicotiana* species.** *Cell Biol Int* 2003, **27:**307–310.
4. Pausheva ZP: **Manual on plant cytology.** Moscow, Kolos Press; 1974, 189–219.
5. Greenberg JT: **Programmed cell death: a way of life for plants.** *Proc Natl Acad Sci USA* 1996, **93:**12094–12097.
6. Caryl AP, Jones GH and Franklin FC: **Dissecting plant meiosis using *Arabidopsis thaliana* mutants.** *J Exp Bot* 2003, **54:**25–38.
7. McCormick S: **Control of male gamethophyte development.** *Plant Cell* 2004, **16:**S142–S153.

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High-scale analysis of pathogenicity determinants of *Potato mop-top virus*

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BMC Plant Biology 2005, **5(Suppl 1):**S39

Potato mop-top virus (PMTV) is the type member of the genus *Pomovirus* characterized by a tripartite, single-stranded, positive-sense RNA genome. However, PMTV differs from the other pomoviruses by having an open reading frame (ORF) for a 8-kDa cysteine-rich protein (CRP) on RNA 3, downstream the triple gene block. Site-directed mutagenesis of infectious cDNA clones of PMTV showed that the CRP ORF is not needed for the systemic infection in *Nicotiana benthamiana* and several other hosts, but it has an effect on the symptom development of PMTV. CRP of PMTV was not capable of RNA silencing suppression in agroinfiltration tests. However, the expression of CRP using vectors based on *Potato virus X* (PVX) or *Tobacco mosaic virus* (TMV) resulted in necrotic symptoms on *N. benthamiana* and tobacco plants, respectively. Sequencing of PMTV isolates obtained from the field revealed significant sequence variability of the CRP. These data suggest that the functions of CRP may be expressed only in certain hosts or at certain phases of the PMTV infection cycle.