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ABSTRACTS
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Experimental Plant Biology. Why not?!

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2.18.

Ultrastructure of *Nicotiana tabacum* cv. Samsun cells infected with CDV

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An emerging virus, Colombian Datura virus (CDV; genus *Potyvirus*), might present a risk to solanaceous crops. It was first identified in ornamental plants (*Brugmansia* spp.) imported from Columbia into the USA in 1967 (Kahn, Bartels, 1968). In 2004, it appeared in field-cropped *Nicotiana tabacum* in three Central European countries: Poland, Germany and Hungary (Schubert et al., 2006).

Nicotiana tabacum cv. Samsun was the model plant mechanically inoculated with Colombian Datura virus. The ultrastructural and cytological studies of infected leaves showed necrotic cells in all types of blades and petioles tissues. CDV particles and cytoplasmatic inclusions were found in the protoplasts of infected cells. They were connected with endoplasmatic reticulum cisternae and/or with mitochondrial external membranes. Often mitochondria were the most changeable organelles. Viral filamentous particles were also present inside nucleus of some infected plant cells. Those localisations were suggested that nucleus, mitochondrion and endoplasmatic reticulum participated in CDV life cycle. Besides, the virus particles and its protein inclusions were detected in floem and xylem elements. This data suggest that all vascular tissues participated in long distance transport of CDV in infected plants.

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2.19.

Localisation of actin and tubulin cytoskeleton in syncytia induced by *Heterodera schachtii* in roots of *Arabidopsis thaliana*

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Cyst nematodes such as *Heterodera schachtii* are obligatory sedentary endoparasites of plant roots. They are able to induce development of syncytium which are large multinucleate feeding site created by fussion of neighbouring cells with initial cell (de Almeida Engler et al., 2004). Syncytium is the main site of molecular interactions between the plant and developing nematode. Characteristic features of syncytia are: hypertrophy of incorporated cells, formation of cell wall openings, proliferation of cytoplasm and decrease of vacuole volume.

The plant cytoskeleton is a highly dynamic and versatile intracellular scaffold composed of microtubules and actin microfilament. It plays important role in many aspects of plant cell growth and development. Both the microlubule and actin cytoskeleton in plants are known to rearange when numerous, diverse external stimuli are applied (Vantard and Blanchoin, 2002).

To find out whether cytoskeleton changes occurred during development of syncytia the immunolocalisation of actin and tubulin was conducted. The microtubule and actin cytoskeletons are concominantly affected in syncytia. Microtubules and actin microfilaments interact with each other structurally and functionally and probably are regulated by common mechanisms.

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2.20.

Novel hosts for transient expression of recombinant proteins among Australian species of *Nicotiana* genus

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Obtaining of foreign proteins from plants by *Agrobacterium*-mediated transient expression has several advantages in comparison with stably transformed plant systems. The main preference is a potential for production of high amounts of foreign proteins during short time. Plant host species may affect strongly the production of recombinant proteins (Sheludko et al., 2007), but at the present time a limited number of species are commonly used for transient expression assays, first of all *Nicotiana benthamiana*. Here we report about novel perspective hosts for transient expression selected among Australian representatives of *Nicotiana* L. genus – *N. excelsior* and *N. cavicola*. We tested these species for accumulation of reporter proteins (green fluorescent protein (GFP), β-glucuronidase (GUS), thermostable lichenase (LicBM3) from *Clostridium thermocellum*) as well as other foreign proteins – desaturases (DesA and DesC) from *Synechocystis* sp. fused with LicBM3 and human interferon α2b (INF).

Plants were infiltrated with *A. tumefaciens* harboring the gene of interest (*gfp*, GUS, *licB*, *desAlicBM3*, *desClicBM3*) under control of 35S CaMV promoter or viral-based transient expression system (Marillonnet et al., 2004) (genes – *gfp*, *inf*). In all experiments the p19 suppressor of PTGS was used to enhance transient expression (Voinnet et al., 2003). The GFP content was calculated by spectrophotometric measurements; enzymatic activities of GUS, LicBM3, DesALicBM3 and DesCLicBM3 were proved using color reactions with substrates; biological activity of INF was demonstrated and calculated using titration method (capability to delay the replication of vesicular stomatitis virus in mammalian cell culture). Total soluble proteins (TSP) were calculated using Bradford method.

The transient expression and accumulation of foreign proteins was observed in all tested systems. Reporter proteins GFP and GUS were successfully expressed in *N. cavicola leaves*. The content of GFP protein was 12.6 ± 6.5% TSP (viral-based system) and 6.0 ± 1.5% TSP (gene under control of 35S CaMV promoter). In *N. excelsior* leaves were successfully expressed *gfp*, *GUS*, *licBM3*, *desAlicBM3*, *desClicBM3* and *inf* genes. The content of GFP protein was 32.1 ± 14.2% TSP (viral-based system) and 3.7 ± 1.7% TSP (gene under control of 35S CaMV promoter). Additionally, biologically active pharmaceutical protein human interferon α2b was successfully expressed in *N. excelsior*. To the best of our knowledge no quantitative data on active IFN α2b

transient expression was reported. The activity of INF was 20.6 ± 7.8 × 10^2 IU/ml (approximate 20-30 ng/g fresh weight) and maximal activity was 32 × 10^2 IU/ml (approximately 30–50 ng/g FW).

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2.21.

Mesembryanthemum crystallinum – *Botrytis cinerea* interaction depends on the photosynthetic metabolism

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Till now, *Mesembryanthemum crystallinum* L. has been studied for its plasticity in the response to abiotic stress factors accelerating the switch from C_3 photosynthesis to Crassulacean Acid Metabolism (CAM), however, the scarce data on the response of C_3/CAM plants to biotic stress are available. *Botrytis cinerea*, a necrotrophic fungus, showed the ability to infect *M. crystallinum* in the C_3 and CAM states. The microscopic inspection of inoculated leaves revealed that the response of C_3 and CAM plants to *B. cinerea* differentiated shortly after inoculation, and in CAM plants the decreased rate of conidia germination in comparison to C_3 plants take place. Detection of response of photosynthetic apparatus to infection with this fungus at 3, 24 and 48h post inoculation was carried out on leaves of different whorls using chlorophyll fluorescence imaging technique. Stimulation of photosynthetic processes after 24h in infected C_3 plants, and just after 3h in CAM plants was observed. The signal induced by pathogen in photochemistry processes of infected leaf was translocated to systemic leaves, before necrotic lesions surrounded by a chlorotic halo developed. The results provide evidence for the involvement of photochemistry processes in the induction of defense mechanisms among the whole plant. Comparing our previous analysis of changes in activities of antioxidative enzymes with measurements of photosynthetic processes, it could be concluded that activation of antioxidative enzymes occurs slower than changes in the photosynthetic processes. Moreover, changes in metabolism of carbohydrates and phenols (the part of non-enzymatic antioxidative system), before induction of enzymatic antioxidants take place. The outcome of plant-pathogen interaction depends on the co-regulation of the photosynthetic mode of carbon assimilation and antioxidative system.

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2.22.

Flavonoid preactivation of *Rhizobium leguminosarum* bv. *trifolii* improves symbiosis with clover (*Trifolium pratense*)

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The *Rhizobium*-legume symbiosis is dependent on the exchange of numerous molecular signals between bacteria and their plant host. Flavonoids secreted by plant roots into rhizosphere play an important role in early steps of symbiosis. They induce the expression of rhizobial *nod* genes, resulting in synthesis of chitolipooligosaccharides (Nod factors). Mixtures of flavonoids may affect survival of rhizobia in the rhizosphere and influence their competitiveness (Cooper, 2004). Nod factors secreted by bacteria elicit multiple responses in the root epidermis that lead to the nodulation of the appropriate host plants (Spaink, 2000).

In this work, we studied the effect of flavonoid preactivation of *Rhizobium leguminosarum* bv. *trifolii* (*Rlt*) on clover nodulation and growth. Three pairs of *Rlt* strains were chosen and clover seedlings were inoculated with mixed cultures of two strains. Four experimental groups were formed for each pair of strains: in I and II group only one strain was flavonoid-preactivated; in III group both strains were flavonoid-preactivated; in IV group none strain was preactivated. For preactivation, the strains before clover inoculation were grown in the presence of clover seeds exudate. Clover plants were grown (4 weeks) under laboratory conditions, then nodule number was estimated and shoots and roots were weighted.

The beneficial effect of flavonoid preactivation of *Rlt* strains on clover growth was observed in the case of preactivation of both strains (III group) and wet mass of roots and shoots was significantly greater in comparison to clover inoculated *Rlt* strains without preactivation. In some cases, the increase of wet root or shoot mass was also visible when only one *Rlt* strain was treated with flavonoids before clover inoculation. Moreover, the increase in nodule number was observed after flavonoid preactivation, but this effect was less pronounced. We concluded, that *Rlt* strains treated with flavonoids, one of a signal factor in rhizobia-plant interactions, may increase the symbiotic efficiency.

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