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on all these data, including the evolution of enzymes within the CYP74 family, we hypothesized the roles of individual CYP74 enzymes in plant life during evolution.
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Roles of CK2 in Auxin Response, F-Actin Organization and Root Phototropism

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Protein kinase CK2 is a well-conserved pleiotropic Ser/Thr kinase. It is a tetrameric protein composed of two alpha (catalytic) and two beta (regulatory) subunits. In plants, CK2 is involved in the regulation of several important pathways, including light signaling and stress-responsive pathways. In order to study the functional implications of CK2 in plants, we have worked with a CK2 dominant negative mutant (overexpressing an inactive catalytic inactive CK2α subunit), previously generated in our laboratory (Moreno-Romero et al., Plant J 2008, 55, 118).
As an alternative strategy of inhibition of enzymatic activity, we have used 4,5,6,7 tetrabromobenzotriazol (TBB), a specific inhibitor of CK2. The loss of CK2 activity revealed interesting phenotypical changes in auxin dependent processes, many of them related with defects in the polar auxin transport (PAT). PAT controls important growth and developmental processes and it is also implicated in responses to directional light sources. We have investigated the gravitropic and phototropic responses in CK2mut plants, and the distribution of auxin during lateral blue light inductions. To do this, we have used the yellow fluorescent protein VENUS fused to the Aux/IAA auxin-interaction domain (DII) and expressed under a constitutive promoter. Auxin has important roles in the reorganization of the actin cytoskeleton. We have investigated F-actin architecture under conditions of CK2 activity depletion, using the GFP-FABD2 F-actin line (a stably transformed line with a fusion construct in which N-terminal GFP is fused to C-terminal half of AtFimA, which includes the second actin-binding domain and the C-terminal end *of A. thaliana* fimbrin 1). We have also studied the effect of phosphatidic acid (PA) on GFP-FABD2 plants after the inhibition of CK2 by TBB. We have found dramatic F-actin depolymerization, due to the loss of CK2 activity, is recovered after PA treatment. PA binds to the capping protein and promotes actin polymerization.
Finally, we have studied also the stability of AUX/IAA proteins, which are repressors of auxin signaling pathways, modulating auxin responses. We have used transgenic plants in which a construct with the AX3 domain fused to GUS (beta-glucuronidase) was placed under the control of the soybean heat-shock promoter HS. Our results show that the inhibition of CK2 activities increases the stability of the AUX/IAA proteins, blocking the activation of auxin-regulated gene transcription.

The effect of brassinosteroids on growth and development of *Phalenopsis* protocorm-like bodies

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Introduction. The content of certain phytohormones and their concentrations in a medium is the determining factor for controlling growth and differentiation of plant cultured *in vitro*. The most commonly used hormones are auxins and cytokinins. Recent studies showed that brassinosteroids (BRs) have a strong modifying effect on growth, development, sex determination and reproduction in higher plants. However these hormones are not studied for their action on growth of plant *in vitro* cultures. Moreover their effects are not investigated in such an important plant as orchids.
The aim of this work was to determine the effect of six different BRs, belonging to two main BR classes, on growth rate and development of *Phalaenopsis* · hybridum Blume protocorm-like bodies. 10^{-10}-10^{-6} M brassinolide (BL), castasterone (CS), epicastasterone (EC), homocastasterone (GC), epibrassinolide (EB) and homobrassinolide (GB) were tested. Culture of protocorms was generated from seeds of *Phalaenopsis* · hybridum Blume. Protocorm-like bodies were isolated from the primary culture and transferred to media containing various levels of BRs. Weigh and length of the protocorm-like bodies were measured after 100 days of cultivation on BR-containing media. Our data demonstrated that all

BRs significantly stimulated orchid growth *in vitro*. The greatest effect on length was caused by CS while maximal increase of weight was induced by BL and EB. Orchid microclones, grown in the presence of 10^{-6} M CS, had twice bigger length that control plants. Weight gain also increased 2 and 3.5 times when plants were cultivated on media containing 10^{-8} M and 10^{-6} M BL, respectively. GB and GC caused smallest effects on growth among all tested BRs. We also compared the BR effects with classical auxins, such as indol-3-acetic acid, indole-3-butyric acid and 2,4-dichlorophenoxyacetic acid. We have found that auxins were less effective than BRs.

Conclusion. We have demonstrated for the first time that BRs stimulate growth of *Phalaenopsis* · hybridum Blume protocorm-like bodies and that this stimulation exceed effect of auxins.

Early cellular events and auxin response during lateral root initiation in the primary root meristem of squash (*Cucurbita pepo*)

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Formation of lateral roots takes place directly in the parental root meristem in several Eudicots plant families such as Cucurbitaceae and Polygonaceae. However, so far there are no reliable data on the earliest events in this type of lateral root initiation neither on the molecular nor on the physiological level. Here, we present data on the earliest events in lateral root initiation in squash (*Cucurbita pepo* L.) and show how organogenesis takes place among proliferating cells.

In order to detect the cellular response to auxin, a new DR5::GFP-GUS-NLS vector was constructed carrying reporter an NLS (Nuclear Localization Signal)-GFP fusion. Another improved DR5::tdTomato-H2B vector containing a *pUBQ10::H2B-Venus* screening cassette was constructed as well. As the tdTomato protein belongs to the brightest fluorescent proteins, the *tdTomato-H2B* fusion allowed detection of even relatively weak responses to auxin in inner layers of the root, and thanks to the use of H2B instead of NLS, even in dividing cells. The *pUBQ10::H2B-Venus* cassette was used to label nuclei and also permitted effective identification of transgenic roots. Composite squash plants harboring these inserts were obtained using a technique developed previously (Ilina et al. 2012 *Annals of Botany*). The nuclear localization of reporter protein allowed a more sensitive detection of the auxin response than cytosolic GFP or GUS. Localization of the first anatomical events in lateral root initiation was carried out on longitudinal root sections (60 μm) by 3D reconstruction of series of optical sections using a confocal laser scanning microscope LSM780 (Zeiss, Germany). Localization of the cellular response to auxin was detected based on the accumulation of GFP or tdTomato in the nuclei of cells.

The cellular response to auxin took place in sister cell pairs (founder cells) directly before the first anticlinal division that initiated the formation of a lateral root primordium in our model system: divisions in the inner and outer layers of pericycle and endodermis. These events took place at a distance 250-300 μm from initial cells. Altogether, cell pairs in two to three longitudinal files of both pericycle layers and one file of the endodermis were involved in lateral root initiation. Later the number of endodermal cell files increased to up to three, and also cells from the inner cortex of the parental root became involved in the formation of the primordium. Remarkably, during the next stages of primordium development only part of proliferating cells maintained the response to auxin. Cells on the periphery of the primordium quickly lost the auxin response. 3D-reconstructions of the earliest events in lateral root initiation and later stages of lateral root primordium development will be presented.

Additional experiments were performed to identify the triggers of lateral root initiation in Cucurbits. Squash seedlings were cultivated in hydroponic culture for four days in the presence of one of growth regulators. It was shown that different forms of exogenous auxins, active auxin transport inhibitors and regulators of ethylene synthesis and signaling had no influence on the number of lateral roots, and thus on lateral root initiation, in our model system.

Our results show that the local increase of the cellular response to auxin in Cucurbits is not required to determine the lateral root initiation site and to maintain cell divisions in the primordium but for determining the direction of the primordium growth and thus, the structure of the lateral root.

Furthermore, data on promoter activity of several meristem-specific genes in squash root tips will be shown and discussed with respect to the influence of these genes on lateral root initiation. Special attention will be paid to WOX5 (meristem-specific transcription factor), SCR (genetic marker of the endodermis), CR4 (receptor-like kinase, regulator of cell proliferation) and ALF4 (Aberrant Lateral Root Formation 4, regulator of the ability to resume the cell proliferation above the elongation zone by lateral root initiation).