07 01

Proteinase inhibitor II is developmentally regulated in *Nicotiana* flowers

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A small protein, NP-PI-II, with an N terminus identical to a part of a *Nicotiana alata* proteinase inhibitor (PI) II, and an inhibitory activity towards trypsin, has been isolated from flowers of *Nicotiana plumbaginifolia*. A larger protein was also present from which the smaller protein is likely derived. In *N. plumbaginifolia* the inhibitor is expressed at very early stages of floral development, this differs from the accumulation pattern during the development of *N. tabacum* flowers. In mature flowers of both species the inhibitor is constituvely expressed at high level in all floral organs. No inhibitor is present in the vegetative meristems and only a low level of inhibitory activity could be induced by wounding leaves. The inhibitory activity remains high throughout the flower maturation and rapidly declines in the ovary after pollination, in fully developed, green, fruit no inhibitory activity could be detected. In mature *N. tabacum* flowers trypsin inhibitory activity is detected extracellularly in the stigma exudate. PI activity is not a regular feature in the stigmas of several Solanaceous genera, this could be related to different strategies concerning fruit-function and subsequent dispersal of seeds. In *Nicotiana* the processing and localization of PI-II appear to be very conserved and PI-II possibly also has a protective function in the flower organs.

07 02

Electric potentials as a factor of morphogenesis and ontogenesis of plants

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1. Electric potentials can be measured in plants with a bright platinum electrode. 2. There are gradients of them in plants. 3. They are dependent on the environment and on the ontogeny of plants. 4. IAA is translocated to the parts having high potentials. 5. CCC, fungicides and other chemicals are translocated to areas of low potentials. 6. Measurements were done in cereals, *Helianthus annuus, Bryophyllum, Pisum* and many other plants. 7. By the distribution of potentials in plant organs the morphology and ontogeny of plants can be explained. Electric potentials support or impede the translocation of IAA or other growth substances from the sites of their production to the other parts of the plant. Under insufficient illumination, for example, electric potentials in the leaves of cereals has a high value, translocation of IAA is enhanced and the leaves grow slender. In cereals, the buds are formed in axils of leaves having high potentials and they grow to the tillers when the potential decreases later on. Therefore, the plant stops to form tillers higher on the stem, because the particular leaves have low potentials and the axial buds are not initiated. It is supposed that the flower stimulation (florigen theory) is not dependent on any chemical compound but it is associated with the high or low potential of a particular plant organ which is transferred.

07 03

Genes that regulate flowering of *Arabidopsis thaliana* in response to daylength

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Our objective is to use *Arabidopsis thaliana* as a model system in which to analyse genes that regulate floral induction. This species is a facultative long day plant, and many mutants showing a disrupted control of flowering in response to daylength have been identified. In general these can be divided into two classes: those that delay flowering under long days but do not affect it under short days and others that cause early flowering under short days. We have examined the genetic interactions between these two types of mutation. In addition we have studied a mutation called *constans* that causes a late-flowering, day-neutral phenotype and examined how it interacts with other mutations affecting gibberellic acid metabolism, phytochrome B activity or floral development. We have cloned the *CONSTANS* gene by chromosome walking, sequenced it and studied its expression pattern. This work suggests that the *CONSTANS* gene product is present under long days and acts to promote the flowering process.

07 04

Plasmalemma signal transduction system and photoperiodic flowering induction of *Spinacia oleracea*

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Major enzymes (i.e phospholipase C and GTP binding protein(s)) of plasmalemma signal transduction system should be regulated by light. A clear regulation of GTP binding proteins by red or blue light exists in different cell systems. This signal transduction pathway implicates calcium as second messenger. This ion has been shown to participate to floral induction of *Pharbitis nil.*

For these reasons, we have investigated this signal transduction chain on leaves plasmalemma during the first hours of flowering photoinduction of *Spinacia oleracea.* We reported here modifications of polyphosphoinositides-phospholipase C activity at the critical photoperiodic time. GTP binding proteins have also been partly characterized and showed concomittant changes. Tentatives to link these two observations by in vitro experiments have failed to show a clear relation between these two proteins.

All these events occurring at leaf level could be propagated to the apical meristem through the symplastic network which is modulated by light and second messengers (i.e inositol triphosphate, diacylglycerol) at plasmodesmata level. These membrane signals should be at the origin of mitosis synchronization, cell determination and finally shoot apical meristem evocation and differentiation.

07 05

The effects of hormones and carbohydrates on growth and flowering of green and SANDOZ herbicides-treated *Chenopodium rubrum* L. plants

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Chenopodium rubrum L. ecotype 184 is a qualitative short-day plant with critical length of the night of eight hours. The medium for *in vitro* culture of green and SANDOZ herbicides-treated plants contained carbohydrates and hormones in different concentrations. Five days after sowing the plants were exposed to non-inductive (15 LD) or inductive (6 SD + 9 LD) photoperiodic conditions. The length of hypocotyl and cotyledon blade were measured and percentage of flowering was scored. The effect of GA_3 on the growth of hypocotyl of green and photobleached plants was stimulatory under SD and inhibitory under LD conditions. IAA showed slight stimulation of hypocotyl growth of green plants only under LD conditions. BAP inhibited hypocotyl growth regardless of photoperiodic regime. The optimal concentration of carbohydrates for green and SANDOZ herbicides-treated plants was 5 % (glucose or sucrose). In green SANDOZ 9785-treated plants exogenous carbohydrates fully compensated for photosynthetic products, but SANDOZ 9789-treated plants needed, in addition, GA_3 in culture medium for 100 % flowering response.

07 06

Floral organ determination

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A shoot meristem is florally determined when it flowers in an environment where vegetative growth would be expected. One way to test for floral determination is to isolate meristems in culture at intervals after the end of the inductive treatment. In the short day plant, *Pharbitis nil*, and in the long day plant, *Lolium temulentum*, the apex forms a complete flower if the apex is detached from the plant after a specific interval following the inductive treatment. Hence, in these cases floral determination is an all-or-nothing-response. We have been studying floral determination in the long day plant, *Silene coli-rosa* using a similar detachment-of-apex-into-culture approach. Our data are consistent with a sequential determination of floral organs in the order that they are initiated in the *Silene* flower: sepals, outer stamens, petals, inner stamens, carpels. The time scale spans several days so that sepal determination ends before carpel determination starts. Moreover, since floral meristems can revert to vegetative growth from each of the floral whorls our data suggest that the floral stimulus is required throughout floral morphogenesis.

07 07

Transductions for pattern formation in flowering: studies in *Helianthus*

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There are many mutations with morphogenetic effects. This means that there must exist a transduction step in expression where the amount of a soluble gene product controls the configuration of solid tissue (including the number of organs to be formed). It is proposed that the pertinent transduction is the physical buckling (folding) of the tunica to accommodate unevenness in the tendency for it to expand. The biological input is uneven growth in the flat plane. The physical output is patterned undulation in three dimensions (floret primordia). This biophysical phenomenon can reproduce the major features of pattern in the sunflower capitulum. Constraint experiments to test this mechanism showed the expected distortion of pattern. Furthermore, they showed that normal buckling was prerequisite to normal gene expression in the floret. In the constrained heads, single bracts replaced the usual dyad floret (bract plus flower). The entire floral part of floret development was eliminated by physical treatment. There is thus transduction from chemistry to physics (buckling) and also from physics to chemistry (gene expression).

07 08

Pollen-pistil interactions in *Petunia hybrida*

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We use petunia as a model system to study the mechanisms which provide for reproductive strategy of species. The biochemical differentiation of male and female tissues is studied and specific proteins with antigenic, lectin and enzyme specificities are identified. It is shown that the function of genetically determined barriers for inbreeding (male sterility, gametophytic self-incompatibility and seedlessness) is due to the operation of a biochemical mechanism in the sporophyte tissue including *de novo* synthesis of proteins with antigenic and enzyme activity and disturbing the complementary interactions at the enzyme substrate level in the pollen-pistil system .

07 09

Physiological signals during floral transition in long-day plants

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Leaves of photoperiodic plants produce promoters of flowering when exposed to favourable daylength regimes. These signals are thought to be transported from leaves to meristems through the phloem, along with the assimilates. Some observations also indicate that the roots possess at least partial control over flowering, presumably through signals transmitted in the xylem with the transpiration stream. Despite many attempts to identify these signals, their precise nature is still controversial. The theory of multifactorial control postulates that the flowering stimulus is a combination of several components (assimilates and known phytohormones). In a number of species, increases in cytokinin levels, most usually in buds and phloem sap, have been detected during floral transition, suggesting that cytokinins could be required. A role as a mitotic stimulus is suggested by the work on *Sinapis alba*, a long day plant inducible by one single LD. In this species, the existence of a shoot to root signal has been postulated, which modifies cytokinin export during floral transition. Another possible signal is sucrose as indicated by recent work on *Sinapis* and other species. A model of signal transduction is proposed which includes interactions between cytokinins and carbohydrates, but also auxin and polyamines.

07 10

Photoperiodic regulation of levels, distribution and metabolism of cytokinins in relation to flower induction in the short day plant *Chenopodium rubrum*

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In leaves, stems and roots of 15-d-old *C. rubrum* plants cytokinin (zeatin (Z), zeatinriboside (ZR), isopentenyladenine (iP) and isopentenyladenosine (iPA)) levels decrease during inductive dark period and increase in light. In apical parts an opposite trend was found- i.e. an increase at the end of darkness followed by a drop in light. R break (15 min in 6th h of darkness) prevented this increase in apical parts. Applied tritiated Z, ZR or iPA accumulated at the end of darkness in much higher level in the apical parts of the plants as compared with plants kept in continuous light. This accumulation is prevented by R break. Flow of cytokinins in xylem and phloem saps was significantly intensified by the dark period and also this change was prevented by R break. The main cytokinin in apical exudates was ZR what indicates that cytokinins flow to the apical part mainly through xylem. Its level is increased at the end of the dark period cca 3 times and is also responsive to R break. The significance of the data for flower induction will be discussed.

07 11

Phenotypic characterization of the recessive mutation *stamenless* (*sl*) in tomato (*Lycopersicon esculentum*)

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The *sl* mutation in tomato consistently affected the floral phenotype causing the homeotic transformation of petals to sepals in whorl two and of stamens to carpels in whorl three. Analysis using histological methods revealed that, at initiation, primordia in whorls two and three of the mutant plants resembled those of the wild type. Divergence occurred during further development when the third whorl organs of *stamenless* fused to the gynoecium which ultimately had an increased locule number and was larger than in the wild type. Growth patterns in the first (true sepals) and second (sepal-like) whorl organs of *stamenless* were not identical: growth was slower and stopped earlier in whorl two. No obvious morphological changes could be seen in other part of the plant.

07 12

Endogenous floral stimulus, state of water and ion homeostasis in relation to flower induction of photoperiodic plants

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Photoperiodic induction of flowering is a complex process of the signal formation which is displayed in changes of the apical morphogenesis. The purpose of our work was to study a number of the plant communication systems (phytohormones, state of water, ion homeostasis) during the flowering induction. Extracts from leaves of induced and flowering SD-plants *Nicotiana tabacum* cv. Maryland Mammoth and *Perilla* were purified using HPLC. Their florigenic activity was studied using bioassays with *Chenopodium rubrum*. It was shown that 15 SD-day induced plants *N. tabacum* and *Perilla* had a florigenic stimulus able to induce the floral morphogenesis in *C. rubrum*. State of water and ion homeostasis in apices and leaves of photoperiodically different biotypes of tobacco plant during the flowering induction were investigated appllying the methods of continuous proton magnetic resonance, NMR spin-echo, atomic absorption spectroscopy. Four fractions of the proton relaxation belonging to water contained in apices of tobacco plants induced for flowering were found (T2 = 0.387 - 0.425 ms; T2 = 3.7 - 3.9 ms; T2 = 10.4 - 13.4 ms; T2 = 20.1 - 23.2 ms). Tissues of organs in vegetative plants were found to have only one component of water with relaxation time characteristic (T2) within the range of 10.4 - 13.4 ms. Induction process involves a sharp increase in the activity of ions (Ca, K) and decrease in that of (Na, Cl) in apices of induced plants. A possibility of functional relation between various plant regulation systems during the flowering induction will be discussed.

07 13

Specific changes in stem extension rate and lipid metabolism upon floral induction in *Chenopodium rubrum*

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Chenopodium rubrum L. plants exhibit an endogenous circadian rhythm in their stem extension rate (SER) in continuous white light. Under continuous blue light conditions SER is characterized by a two-frequency oscillation, that can be explained by the existence of two endogenous oscillators. The higher frequency oscillation under these conditions corresponds to the oscillation in SER of defoliated plants. Thus the higher frequency oscillation is supposed to be located in the stem or apex, while the lower frequency oscillation might originate in the leaves. On the other hand intact flowering plants show, in contrast to vegetative plants, a significantly shorter period length in SER. Thus the resulting SER-pattern could be considered as an interaction between a leaf and a stem/apex oscillator. Changing the frequency of the leaf-based oscillation by an appropriate environmental signal results in a modified interaction between two frequency coded signals of leaves and the shoot (apex) oscillators and possibly provides the information needed to induce flower formation at the apex. Considering that modifications of membrane composition and changes of membrane state might be involved in the clock mechanism and in the mechanism of flowering, we compared kinetics of the relative amount of fatty acids in vegetative and flower-induced plants. Simultaneously recorded SER-kinetics were correlated with rhythmic fluctuations in fatty acid composition of stems and leaves.

07 14

Flowering induction of tobacco plants and cytokinin content

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According to the general concept cytokinins form one of the flowering control systems. Studies on various types of elements of the photoperiod-sensitive plants (SDP *Chenopodium rubrum* and *Xanthium strumarium*, LDP *Sinapis alba*) showed a correlation between the cytokinin content in different plant organs and flowering induction that allowed to suggest a possibility of photoperiodic control of those compounds level and their involvement in induction processes. The purpose of this work is to determine the cytokinin content in tobacco plants with different photoperiodic sensitivity (SDP *Nicotiana tabacum* Maryland Mammoth and LDP *Nicotiana silvestris*) in leaves of various nodes during the flowering induction. By the HPLC purification using reverse phase C18 columns as well as by bioassays there was analyzed the content of zeatin (Z), zeatin riboside (ZR), isopentenyladenine (IP), isopentenyladenosine (IPa), benzylaminopurine (BAP) and benzylaminopurine riboside (BAP-R). The results obtained showed the absence of correlation between Z and ZR and flowering induction in LDP *N. silvestris* as well as some increase in Z content, as compared to the control, in leaves of the upper and lower nodes in SDP *N. tabacum* after 10 SD induction. Some increase in BAP and IPa content was found to occur in leaves of different nodes in both types of plants following 10 and 15 days of induction that allowed to suggest a possible involvement of those phytohormones in flowering induction processes.

07 15

Histochemical studies of English walnut *(Juglans regia* L.) flowers morphogenesis

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During all morphological differentiation stages of English Walnut (*Juglans regia* L.) female (pistillate) flowers distribution regularity of proteins, insoluble polysaccharides and enzymes is analogous to that observed in male flowers. Localization of substances and their maximal reaction intensity coincides with the periods of initiations, intensive growth and development of embryonal structures. In both flower types vascular bundles and adjacent tissue sections are more responsive to histochemical reactions, especially to peroxidase and ascorbic acid. Starch is almost undetected, whereas insoluble polysaccharides are present in embryonal organs of differentiating male and female flowers in two forms: as granules and in dispersal state. Unlike staminate flowers, whose metabolism is intensified twice during differentiation (in the year of initiation and in the flowering year) the pistillate ones display only a spring peak

07 16

Process of vernalisation in chromosome substituted wheat cultivars

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The *Triticum aestivum L.*cultivars Chinese Spring 42 (recipient) and Cheyenne (donor) were vernalised together with the substitution lines CS(Ch5A) and CS(Ch7A). The abscisic acid and polyamine contents, as well as the activity of different hydrolytic enzymes (RNase, invertase, amylase) were studied in the leaves, crowns and roots of the cultivars. A remarkable increase was observed in the abscisic acid and polyamine contents during the first period of cold treatment, which slowly decreased to the initial level by the end of the first ten days. After that a slight increase could be observed from the 40th day till the end of vernalisation. The changes in the activities of hydrolytic enzymes exhibited similar patterns, usully giving the highest values at the crowns. The enzymatic activities and polyamine and ABA contents of the substitution lines resembled the recipient Chinese Spring 42 values rather than those of the donor Cheyenne. Changes in the physiological parameters of wheats from the 40th day on are probably due to a slight increase in gibberellic acid content.

07 17

The mechanism of calcium action on flower induction in *Pharbitis nil*

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Calcium ions, calcium ionophores, as well as caffeine, stimulated the flowering of *Pharbitis nil* when applied just before and 2 h after the start of a subinductive, 14-h, long dark period. Their application on the cotyledons between 6 and 10 h after the start of the dark periods results in an inhibition of flowering. It is proposed that stomata are the targets for calcium and calcium modulator action. It was found that in the middle of the dark period, extrusion of an unknown material through the stomata occurred. This material is synthesized inside globular bodies which are equally distributed throughout the cotyledons. Using a confocal scanning laser microscope and the calcium fluorescence indicator, Fluo-3, free calcium has been found inside these structures. It has been shown that the level of free calcium increases inside their matrix during the inductive dark period. By means of GC-MS it was shown that changes of calcium level inside the bodies are correlated with changes in the phenolic content of their matrix. The role of the bodies in calcium-controlled stomatal movement as well as calcium-dependent regulation of flowering will be discussed.