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Regulation of lateral root development

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Bakalářská práce

Regulace vývoje postranních kořenů

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I declare that my presented work has been developed independently using cited literature led by Dr. Aleš Soukup, and that has not been presented as a bachelor thesis at any other university.

In Prague, August 2009 Alois Hilgert

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List of abbreviations

LR	Lateral root
LRI	Lateral root initiation
LRP	Lateral root primordium
BR	Brassinosteroid
BL	Brassinolide
RH cells	Root hair cells
NH cells	Non root hair cells
QC	Quiescent center
LAX	Like AUX, influx carriers
OsCKI	Casein kinase 1 gene
RSA	Root system architecture
KRP2	Kip-Related Protein2 krp2
GSA	gravitropic set-point angle
IL	Inner layer
OL	Outer layer (OL)
VFB	VIER F-BOX PROTEINE (VFB)
CEG	CEGENDUO
PIN	Auxin efflux carrier complexes
IAA, 2,4-D, NAA	Auxins
ABA	Abscisic acid
CK	Cytokinin
GA	Gibberellic Acid

AIR3, PG,XTR6	Cell-wall-modelling genes
AXR4	Auxin resistant mutants
ARF	Auxin response factors
SLR	A member of Aux/IAA auxin/response family of proteins
VSP29	Membrane trafficking component
E1, E2, E3,…	Ubiquitin protein
SCF^{TIR1}	Mediates AUX/IAA degradation
DBD	DNA binding domain
ALF4	Aberrant lateral root formation
AHK	*Arabidopsis* histidine-kinases
ABI	ABA INSENSITIVE
ERA1	Enhanced response to ABA1
NPA	N- phtalamic acid
DR5::GUS	Auxin-inducible promoter in root tips
AtPCT1	Membrane carnitine transporter
CLAVATA1/HAR1	Putative receptor kinases
NIT3	Nitrilases convert indole 3 acetonitrile to IAA
MYB77	Transcription factor involved in auxin responses
MADs Box	Homeotic genes

Abstract

Plant roots are required for the acquisition of water and nutrients, for responses to abiotic and biotic signals in the soil, and to anchor the plants in the ground (Nibau, C. et al. 2008). Plant morphology is dramatically influenced by environmental signals. The growth and development of the root system is an excellent example of this developmental plasticity. Both the number and placement of lateral roots are highly responsive to nutritional cues. This indicates that there must be a signal transduction pathway that interprets complex environmental conditions and makes the "decision" to form a lateral root at a particular time and place (Malamy et al. 2001).

The signalling pathways triggering these modifications remain mostly obscure(Nacry, P. et al. 2005). In spite of that, many specific regulators related to the sensing of the external signals and internal status of the plant have been discovered, this increase the evidence that phytohormones play a key role in mediating complex pathways of plant responses. New studies about how these mechanisms work in the plant and the interaction between many phytohormones elucidate our understanding in plant physiology.

This research thus, shows an overview of the basic root anatomy in some plants, and then gives a summary of most lateral root developmental interactions.

Key words: Lateral roots; Environmental signals; abiotic signals; biotic signals; developmental plasticity; nutritional cues; phytohormones; root system; growth and development of plants

Abstrakt

Kořeny rostlin jsou potřebné k získávání vody a živin, k odpovědi na abiotické a biotické signály půdy a pro zakotvení rostlin v zemi (Nibau, C. et al. 2008). Morfologie rostlin je výrazně ovlivněna environmentálními signály. Růst a vývoj kořenového systému, je vynikajícím příkladem vývojové plasticity rostlin. Umístění i počet bočních kořenů jsou vysoce závislé na přísunu živin. Z toho vyplývá, že musí existovat signální dráhy, které na základě okolních podmínek určí, kdy a kde se vytvoří postranní kořeny (Malamy et al. 2001).

Ačkoliv signální dráhy procesu růstu laterálních kořenů jsou ještě málo známy (Nacry, P et al. 2005), bylo objeveno mnoho specifických regulátorů spojených s vnímáním vnějších podnětů či vnitřního stavu rostliny. Nabývá důkazů, že fytohormony hrají klíčovou roli ve zprostředkování souboru drah roslinných odpovědí. Nové studie mechanismu těchto reakčních cest a interakcí mezi fytohormony, rozjasňují naše porozumění roslinné fyziologii.

Tato rešerše dává přehlédnout základům anatomie některých rostlin a shrnuje současné poznatky o interakcích vývoje postranních kořenů.

Klíčová slova: Boční kořeny, podněty vnějšího prostředí, abiotické signály, biotické signály, dostupnost živin, vývojová plasticita, fytohormony, kořenový systém, růst a vývoj rostlin.

I. Introduction

Unlike animals, higher plants generally develop most of their organs postembryonally throughout the whole lifespan. In the aerial shoots, the meristems produce leaves, stems and floral organs, which are initiated on the flanks of the meristem (Fukaki, H. et al. 2007). Underground, a branching root system develops through the production of many lateral and adventitious roots from the internal tissues of the parental roots and shoots (Charlton WA. 1996). Certain cells can be activated to produce new shoots and roots, each with a new population of meristematic stem cells at the tips. Plants are completely dependent on the resources that are available in their immediate vicinity. Unfortunately, nutrient availability and distribution are in constant flux in the environment. Plants must be able to sense these changes and respond appropriately. The presence of active stem cell populations and the ability to generate new cells and tissues allows the plant to adapt its morphology to its unique and changeable environment(Malamy, J. E. and Ryan, K. S. 2001) In particular, the availability of nutrients affects both the number and location of lateral root initiation sites (Drew, M. C. 1975, Drew, M. C. and Saker, L. R. 1978, Drew, M. C. and Saker, L. R. 1975).

It is important to understand that because plants have a totally different anatomy, development and way of living is logical, that their metabolism, signalling and responses to their environment, will differ to those in animals in many manners.

The plants world is governed by several factors that have to be elegantly sensed in order to adapt them to fast-changing surrounding conditions. Developmental plasticity provides a wide range of advantages to the plant, allowing it to collect signals and information from its environment and incorporate them into decisions about growth and development. This allows otherwise immobile plants to place structures in the optimal position with respect to water, nutrients, and sunlight. Nevertheless, it is logic that, developmental plasticity also poses certain risks. It requires that certain cells in the plant remain undifferentiated, or able to de-differentiate to form stem cell populations. This in turn presents the danger of overgrowth or unorganized growth, an equivalent of animal cancers(Malamy, J. E. 2005). Thus, important highly controlled mechanisms of stimulation and suppression must be ridden in the plant. The root system, the lateral root system specially, is a fabulous place to study the determinants

of architecture, and of developmental plasticity. Improving the natural responses, uptake effectiveness and root system architecture (RSA), we will be able to generate a more efficient and more profitable nutritional field needed due to our fast-growing human population.

II. REVIEW OF LITERATURE

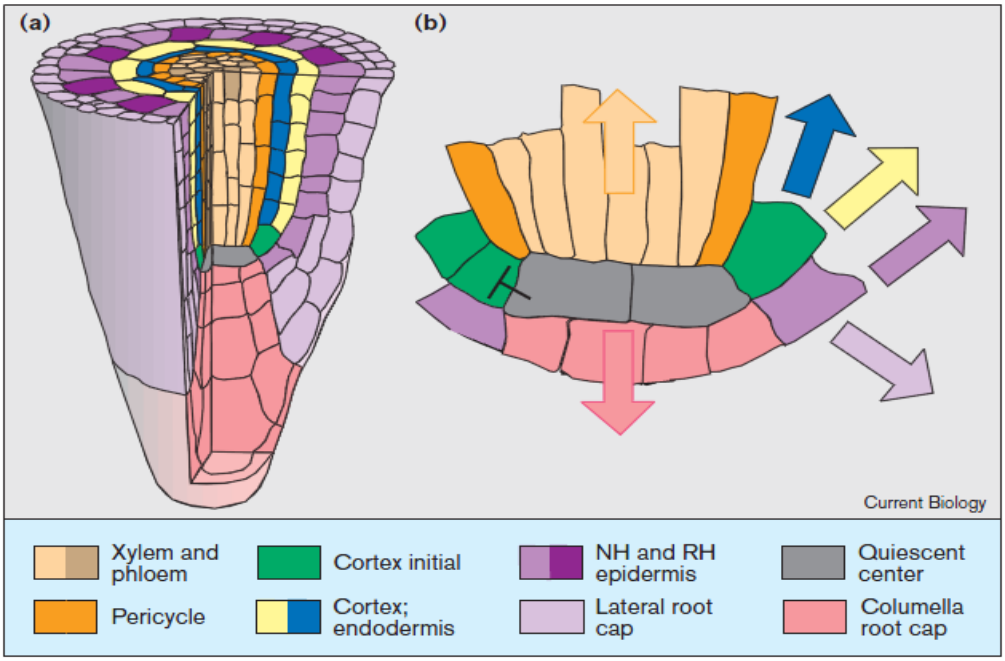
2.1The Plants Anatomy:

2.1.1. Main root characteristics

The first root of the seed plants or primary root develops from the apical meristem at the root end of the embryo. Structure of the root system varies considerably among plant taxons. The root system of gymnosperms and dicotyledons is formed mostly by the main root and lateral roots, while the root system of monocotyledons is dominated by adventitious roots. Main root often lasts only a limited time or stops its growth and the root system is dominated with adventitious roots growing from the stem. The most important functions of the root system are absorption of water and mineral compounds, anchorage in substrate, storage, transport, communication, and metabolism of amino acids, alkaloids and growth regulators among others. There are many types of roots which are specialized to particular function. For example in vines and epiphytic plants in the manner of grow or the tuberous roots or storage roots like Sweet Potato (Ipomoea batatas) or Dahlia, used a lot by Aztecs, also Cassava which is the third largest source of carbohydrates for human food in the world, with Africa its largest center of production [1].

The internal organization of the root, is variable but in general looks simpler and structurally also looks more primitive than that of the stem because shoot meristems form lateral organs exogenously alterning nodes and internodes. The root has no leaves like organs and with no division into nodes and internodes, no stomata. The arrangement of tissues in the root shows relatively little difference from level to level in the vertical plane. Its structure is axial. Instead, in the stem, the connection of axis with the leaves results in different structures between nodes and internodes and even between different levels of a given internode.

We can distinguish different root regions along the longitudinal axis: the root cap covering the tip, the root meristem and the quiescent center, distal transition zone, the elongation zone and the differentiation zone. Also in a transverse section we distinguish three basic tissue systems: dermal tissue system of the epidermis with root hairs, the cortex (ground tissue system), and the vascular tissue system including the surrounding pericycle. Each tissue system has some structural features that are characteristic for roots. The innermost layer of cortex is always differentiated as endodermis and the layer/layers below the epidermis are mostly differentiated as exodermis. The centre of the root is occupied by central cylinder containing vascular tissues and pericycle which is located at the periphery of the central cylinder (Esau, K. 1965, Katherine Esau 1965)



Cell fate in the *Arabidopsis* root meristem. **(a)** Cell types. **(b)** Stem cells (initials) and their direction of cell division.

Fig. 1 (Benfey, Philip N. and Scheres, Ben 2000)

The apical meristems structure and function, which are under the control of regulatory mechanisms, give rise to an organized root system. By its analysis from the embryo to a mature root, it´s possible to trace out planes of cell divisions and the direction of growth from which each tissue is formed. In one analysis, the parts of differentiation are followed to the apex of the root to determinate whether there are specific cells that

appear to be the source of one or more tissues .There is an explicit relation in which, the apical cells function as initials of the other groups of tissues. In seed plants, the root presents two principal pattern of the spatial relation between the tissues and the cells in the apex. In the first, the vascular cylinder, the cortex, and the rootcap, each, may be recognizable in an independent tier of cells in the apical meristem, with the epidermis differentiating from the outermost layer of the cortex or depending of the plant, having a main origin with the rootcap, typical for dicots, each have their own initials; we say to this close type of apical organization (Esau, K. 1965, Schade, Christiane and von Guttenberg, Hermann 1951In). In the second type, all the regions or at least the cortex and the rootcap converge in one group of cells transversally oriented, all regions have a common group of initials; this last type is the open type of organization, thought as physiologically more primitive (Esau, K. 1965). This open type organization is typical for gymnosperms, being seen often in dicots while the closed type is typical for monocots. However both types can be found in each group; as for example in monocots, its typical the close type, most of grasses, but in onion, Allium cepa it is opened. In many lower vascular plants, only one cell, the apical cell, is the common initial for all parts in the root (Esau, K. 1965). In dicotyledons closed and open schemes are commonly believed to remain unchanged during the growth of the root axis. In some roots was proved that early growth is followed by deceleration, after which the initial cells stop dividing, elongation ceases, and the root reaches its determinate length. At or before reaching determinacy, the root apical meristem stops maintaining its closed organization and becomes less organized(K.Chapman, E. P. Groot S. A. Nichol and T. L. Rost 2003).

Seems that in some species, lateral roots can after a short period of time get more organized, in Typha was seen an special phenomenon in which, the newly emerged LR apical meristem produces and open configuration with cells derived from the ground meristem/protoderm (cells differentiating to covering tissues), and calyptrogen (the initial layer of the rootcap), then however, after getting longer, most lateral roots change to again closed or three-tiered meristem(Seago, J. L. and MARSH, L. C. 1990).

Initials of the root apical meristem (undifferentiated actively dividing group of cells at the tip of the root from which new cells are formed are located around a non-dividing

set of cells which form the quiescent center (QC). Quiescent center cells have a characteristic ultrastructure and express distinct genes. It was seen that laser ablation of QC cells intensifies the differentiation in the nearest initials, suggesting that the QC keeps the initials in an undifferentiated stage(Benfey, P. N. 2005).

Many works studying root differentiation use *Arabidopsis thaliana* as a model plant because of the simple structure of its primary root. We distinguish several tissues in the root of A. Thaliana, still the most studied model plant. The four layers; epidermis, outer cortex, endodermis and pericycle (already part of the vascular cylinder) surround the vascular tissue in the center of the root. The outer part of the epidermis is formed by two types of cells, the ones which form root hairs (RH cells) and others that don´t (non-hair or NH cells). Root hair cells are able to form hairs already close to the root tip, just behind the region of root elongation where the first conducting elements mature. In some plants the root hairs only arise from specialized cells called trichoblasts. Root hairs usually do not last more than several days, but the surface area of all the plant´s root hairs greatly increases the total root surface that functions in the absorption.

In the endodermis, each endodermal cell has a Casparian strip which is a continuous band around both radial and transverse walls impregnated with suberin and lignin. Casparian strips are forming an apoplastic barrier to movement of water and solutes within apoplast. Casparian strips, appears in the region of maturation of the first xylem elements. The presence of endodermis permits the plant to uptake selectively the compounds it transported farther not through apoplast but through the symplast, entering the cytoplasm, because of selective transport through the plasmamembrane, in order to reach the transport elements in the vascular cylinder. In most species, the endodermal cells develop further to a secondary state which involves the deposition of thin suberin lamella over the entire inner surface of the wall, which can be followed by the inward deposition of lignified cellulosic secondary wall, the tertiary state (Pazourek J. and Votrubova O. 1997).

The vascular cylinder appears as a column in the center of the root, and is derived from the procambium. Inside the core, vascular tissues are differentiated forming

different patterns (diarch, triarch, etc.) In A. Thaliana, a diarch arrangement is found. In spite of fact that the protoxylem cells mature first, the metaxylem exceeds the protoxylem cells in width and therefore is more obvious at the onset of enlargement and vacuolization. The primary xylem differentiates centripetally and is exarch (the older elements are in the outermost part of the tissue). Phloem differentiates in the same direction, that is, centripetally. Thus first, the protophloem appears next to the pericycle, then, the metaphloem deeper in the vascular core.

This sequence of xylem differentiation described above is common in both monocotyledons and dicotyledons (Esau, K. 1965, Pophan, R. A. 1955, Riopel, J. L. 1964) In Esau, k. 1965). In some plants (e.g. Cucurbita pepo) the central part of vascular cylinder remains as parenchyma for long time. Then several centimetres from the apex, one cell from the pith center increase about four times in diameter and differentiates as a metaxylem vessel member, causing in the process a considerable rearrangement of the surrounding cells (Harrison-Murray, 1973; Hayward, 1938) reviewed in (Esau, K. 1965).

The longitudinal differentiation of the primary vascular tissues in the root is acropetal (from the base toward the tip), in which the protophloem is maturing closer to the apical meristem than the first xylem (protoxylem) (Esau, K. 1965, Katherine Esau 1965)

The distances between the apex and the first matured vascular elements, especially those of the xylem, vary (Esau, K. 1965). They are affected by age of root, rate of growth, plant species, presence of a disease or stress, type of root (short or long type, terminal or lateral), and other factors (Peterson, R. L. 1967, Riopel, J. L. 1964, Seago, J. L.). In general, the mature vascular elements are significantly closer to the apical meristem in slowly growing roots than in rapidly growing roots. The proximity of mature vascular tissues to the apex can change in the same root under different environmental conditions. During dormancy of perennial plants, for example, root growth decelerates and the maturation of vascular cells advances toward the apex (Esau, K. 1965).

2.1.2. An introduction to Lateral Roots

In this research my commitment is to review external factors and internal regulatory mechanisms that take part in the process of forming the root system architecture (RSA), and how these mechanisms are working in diverse plants, especially in lateral roots development.

Important part and very variable of the root system are the lateral roots (In the text further as LR), LRs emerge from the periphery of the vascular cylinder at different distances from the apical meristem. As their origin is from deep tissues within the root and not from the surface, LRs origin is known to be endogenous.

Depending on the rank of the root the gives rise to laterals, the latter are frequently designated as secondary roots, tertiary roots, and so on (Esau, K. 1965).

Most commonly, the LR of gymnosperms and angiosperms, emerging on main roots or their branches, or in adventitious roots, are originated in the pericycle (Lloret, P. G. et al. 1989). But we can't generalize this if we include ferns, in which, on the contrary, lateral roots originate from the endodermis.

Many similarities are found between anatomical features and development of lateral roots of various species. However, exist environmental factors, species specific traits and ontogenic variability of individuals that must be taken into account. From this point, the well known simple anatomy of *Arabidopsis thaliana* has been exhaustively studied but also compared to other model plants.

2.1.3. The pericycle:

The pericycle is the outermost layer of the vascular tissues. The pericyclic origin of the lateral root places is in close juxtaposition with the vascular tissues of the parent root to which the vascular tissues of the lateral root connect. The position of the lateral root with regard to xylem ridges of the parent root varies in relation to the vascular pattern of the parent root according to some species but is stable in a root of

specific plant species. All cells in the pericycle are in theory capable of lateral root initiation, but under normal circumstances only the pericycle cells nearest the internal xylem or phloem poles, depending on the plant species, perform this function (Benfey, P. N. 2005). In general, mostly in a diarch root, the lateral root often arises between phloem and xylem, in a triarch, tetrarch, and so forth, opposite the xylem, in a polyarch monocotyledon root, opposite the phloem.

In *Arabidopsis*, Lateral root (LR) primordia originate from a subset of pericycle cells which undergo asymmetric divisions as founder cells. These founder cells give rise to LR tissues by clonal expansion, the undifferentiated cell divides in two; one stays the same as the parent and the second acquire a developmental fate different from that of their mother and, as a consequence, play a principal role during the first stages of lateral root initiation(Dubrovsky, J. G. et al. 2001).

Lateral root development in front of the phloem is seen mostly in monocots like *Zea mays, Secale, Hordeum* (barley), *Triticum* (wheat), nonetheless this phenomenon was found even in dicotyledon carrot (Esau, K. 1965, Lloret, P. G. et al. 1989). However, in monocots, mostly with polyarch pattern, this description results relative since the LR primordium bridges neighbouring xylem parts.

In dicotyledons plants, such as *Arabidopsis*, *Helianthus annuus* (sunflower), *Raphanus sativus* (radish), *Pisum sativum* (pea), *Lactuca sativa* (lettuce) the LR development is found in front of the xylem, although this type of architecture is seen in the monocotyledon Allium cepa (onion) as well (Esau, K. 1965, Laskowski, M. J. et al. 1995, Lloret, P. G. et al. 1989).

Lateral root primordium originates from a subset of the pre-defined pericycle cells. Within this population of cells group of cells is elected, so called founder cells, which establish the whole lateral root. The estimated number of founder cells varies among species and is sometimes inconsistent for different authors. The number of lateral root founder cells in Vicia faba was estimated to be 24(Davidson, D. 1965). In another study, the range of founder cell numbers was calculated to be between 12 and 162, depending on the species ((MacLeod, Ronald D. and McLachlan, Sandra M. 1975). The number given for Vicia faba, 162, was based on the assumption that primordium initiation takes place well behind the root apical meristem. Using direct histological

observation, the number of founder cells in radish roots was estimated to be 30 (Blakely, L. M. et al. 1982). In *Arabidopsis*, were made histological observations to estimate the number of founder cells, taking into account the average length of the phloem-radius pericycle cells, and assuming that the cell files that lie between the xylem-radius and phloem-radius, cell files are intermediate in length, it have been estimated that the average number of founder cells in an *Arabidopsis* lateral root is about 11(Laskowski, M. J. et al. 1995).

The endodermis also can contribute to the root primordium with some cell layers. Not often happens that, the cells of endodermal origin are sloughed off after the lateral emerges from the parent root (Byrne, J. M. 1973, Esau, K. 1965). Sometimes the pericycle doesn't create lateral root initiations all alone but it is assisted by the endodermis in some plants(Seago, J. L. and MARSH, L. C. 1990).

In ferns, as seen in Ceratopteris, the first sign of LR initiation was cell expansion in the endodermal layer. This enlarged cell was designated as the lateral root mother cell that give rise to LR primordium (Hou GC, Hill JP Blancaflor EB 2004).

The initiation and growth of LR follows acropetal sequence (Dubrovsky et. al 2006) with the youngest primordia found the closest to the root tip. However further development might be arrested and early developmental stages might be found among growing LRs. In some cases, the competence of the parental root pericycle or endodermis to initiate LR primordia extends along broader regions of the parent root and new primordia can be initiated in the zone of already established LR primordia, like in *Arabidopsis Thaliana* (Dubrovsky, J. G. et al. 2006).

The formation of LR´s in an inverse order to the natural is unusual. For example, basipetal (from the tip toward the base) sequence of LR development occurred on the preformed root regions in the germinative radicle of *Theobroma cacao* but from its origin we can say they could be rather adventitious. (Lloret, P. G. et al. 1989)

The existence of dormant or late-formed LR primordia presents a problem to predict an average distance at which new initiation events should occur in a growing root (Dubrovsky, J. G. et al. 2006).

Variation of pericycle structure and LR positioning can be shown within roots of *Allium cepa, Pisum sativus,* and *Daucus carota.* In the central cylinder of pea, P. sativum, the triarch primary root has a single-layered pericycle in the vicinity of phloem poles and two- or three-cell-layered pericycle opposite the xylem poles; while the onion adventitious root tends to be pentarch, being bounded by a single-layered pericycle. In grasses is seen that the pericycle can be interrupted in front of the protoxylem and its continuity is thus, not maintained. In carrot, cells of the outer pericycle were measured as shorter than those of the inner pericycle (two cell layers), and both groups of cells were markedly shorter than those opposite the phloem, similarly was in onion. Significant differences in the endodermis between lengths of opposite xylem and opposite phloem cells were founded, while there were not significant differences between the two locations in the cortex. The first steps in LR development are similar in onion; A. cepa and the pea (see section 2.2.1). In *P. sativum* the cortical cells also undergoes mitosis contributing cells to the primordium.(Lloret, P. G. et al. 1989)

While monocotyledon onion has just one-cell-layered pericycle (LR develops near the xylem, not really usual in monocots), dicotyledons carrot (LR develops in front of the phloem) and dicotyledons pea (which *as A. thaliana* and radish has LR initiations near the xylem) have in the areas of respective LR initiation; phloem or xylem, a pericycle multi-cell-layered (Lloret et al. 1989).

Lateral Root Primordia(LRP) tissues were mainly derived from the pericycle with endodermal cells forming a thin overlying layer (Lloret, P. G. et al. 1989). The pericycle in carrot also contains two cell populations which can be clearly distinguished on the basis of mean cell length. In contrast to P. sativum and A. cepa, the shortest pericyclic cells are those adjacent to the phloem. In the endodermis, smaller differences in cell length are recorded.

The middle cortex shows no significant differences in mean cell length between cells opposite xylem and opposite phloem in the three species, suggesting that two distinct populations arise only in those tissues directly involved in LR formation.(Lloret, P. G. et al. 1989)

In Allium cepa, the cells of the pericycle that divide periclinally are very short. This is appreciably in plants in which the first periclinal divisions happen far from the tip. In these plants, all the pericycle cells become elongated and highly vacuolated before the lateral root initiation starts (Casero, P. J. et al. 1995). Mitoses proceed via unequal divisions. Often such divisions are coordinated in adjacent cells.- Thus, after the divisions, cluster of short cells of the pericycle (founder cells) are observed in front of the xylem, and generally, are the first which divide periclinally. In carrot roots, wheat roots and maize roots, as well this pair of cells always is seen near the phloem. (Casero, P. J. et al. 1995, Casero, P. J. et al. 1993, Demchenko, N. P. and Demchenko, K. N. 2001)

The short pericycle cells, which divide periclinally during lateral root development, are the product of asymmetrical transverse divisions. Furthermore, the first asymmetrical division always occurs in the same topographical location relative to the vascular pattern as the lateral roots. This tells us that asymmetrical transverse divisions are the earliest stage in the development of the LR, continued then by periclinal divisions. Similar transverse divisions happen in the endodermis cells in ferns (Clowes, 1961, Liu and Raghvan, 1991). The generalization of the phenomenon shows that LR formation is another important example of development in plants where change of cellular fate is connected with asymmetrical divisions. Prior to these asymmetrical transverse divisions, it must to be summed that the nucleus moves towards the end of the cell when a pericycle cell is activated to initiate a lateral root primordia (LRP) (Casero et al. 1995). Variety of other developmental processes, including root hair (avers, 1963), stomata (Stebbins and Jain. 1960), as well as LR formation (Bell and Mc Cully, 1970) make use of asymmetrical division. On the other hand, in the *Pisum* pericycle the transverse divisions are described as consistently symmetrical (Lloret, P. G. et al. 1989). Results from these authors suggest that in regions of *Pisum* roots far from the apex, the mitotic activity restarts again in a controlled process of cell life cycle. The conclusion was that mitoses cease beyond the apex and then restart as the cells approach the level of LRP initiation. But how this happens? When? As we will see, many factors, including phytohormones down and up regulate these processes. LR initiation could be an excellent model with which study cell-cell interactions during development and differentiation in plants.

The pericycle cells which are involved in the lateral root initiation would form a symplastically isolated group of cells in which a sequence of divisions occur from the contiguous end of one cell to the other(Casero, P. et al. 1996). The authors observed that the short cells then, realize periclinal divisions following the same polarization. Simultaneously, the mother pericycle cells undergo an asymmetrical radial expansion. Even though each mother pericycle cell follows this sequence of divisions, this not mean that they all have the same capacity to divide, this capacity is being loosing in the way they get closer to the periphery, and so, while all of the short daughter cells from the central pair of the mother pericycle cells divide periclinally, the most peripheral mother pericycle cells undergo only one or two transverse divisions (Casero, P. et al. 1996).

2.2. Lateral Roots

We define lateral roots as those that originate from pericycle of some other root. This means that lateral roots can be derived either from a seminal root, an adventitious root, or another lateral root. They are important for the plants to adapt to the environment and use its resources (Lloret, P. G. et al. 1989). Development and growth of existing roots and initiation of new lateral roots is considered to be an important feature increasing absorbing surfaces, and bringing these surfaces into contact with new undepleted areas of soil. The distribution of the lateral roots along the parent roots is not at random and is related to the environmental conditions, allowing optimal utilization of soil resources (Lloret, P. C. et al. 1998, Lloret, P. G. et al. 1989). Regulation of lateral root (LR) development in higher plants is very complex, interesting and our knowledge of this area is significantly incomplete. Basic outlines of interlinked regulatory pathways were sketched (Fukaki, H. et al. 2007).

When a lateral root is initiated, several contiguous pericyclic cells acquire dense cytoplasm and divide periclinally. The products of these divisions divide again, periclinally and anticlinally. The accumulating cells form a protrusion, the root primordium. And as the primordium increases in length, it penetrates the cortex and emerges to the surface. The parent tissues adjacent to the lateral roots can undergo major structural and functional changes to facilitate the growth of the lateral root inside parent root and its emergence(Lloret, P. G. et al. 1989).

At the site of origin of a lateral root the cells of the parent root are variously affected by the new growth. If the lateral root arises close to the apical meristem, the parent root cells are not yet fully differentiated and initiate the meristematic activity with no profound histologic changes. If the endodermis has Casparian strips where the laterals arise, it usually divides in front of the proliferating pericycle and may form Casparian strips in the new cells, at least for a time. Lateral roots, however, may arise also at root levels where the pericycle and the endodermis have lignified secondary walls (Bell, J. K. and McCully, Margaret E. 1970, Karas, I. and McCully, M. E. 1973). It is possible that delignification and removal of secondary walls precede the meristematic activity concerned with the initiation of the lateral root (see section emergence 2.2.2).

Vascular tissue of the newly formed lateral roots is connected to maternal root tissue. When phloem and xylem begin to differentiate in the lateral root, after emergence, these tissues become connected with the equivalent tissues in the parent root by a differentiation of the intervening parenchyma cells into vascular elements. Symplastic connection of two phloem system takes place (Oparka, K. J. et al. 1995). These parenchyma cells are first of all those derived from the pericycle derived primordium tissues. Depending on the extent of vascular connection to be established, cells in the parent vascular cylinder beneath the pericycle may also participate by division and vascular differentiation in forming the connection(Bell, J. K. and McCully, Margaret E. 1970, Esau, K. 1965).

2.2.1 Developmental stages of lateral root formation

There are many reviews on the process of lateral root development from the parent root. The main model is *Arabidopsis* in which, as in most eudicots, LR initiation occurs in pericycle cell files adjacent to a protoxylem pole of the stele. The process has been divided into 8 stages (Stage I-VII and Emergence), as described in detail Malamy and Benfey, 1997.

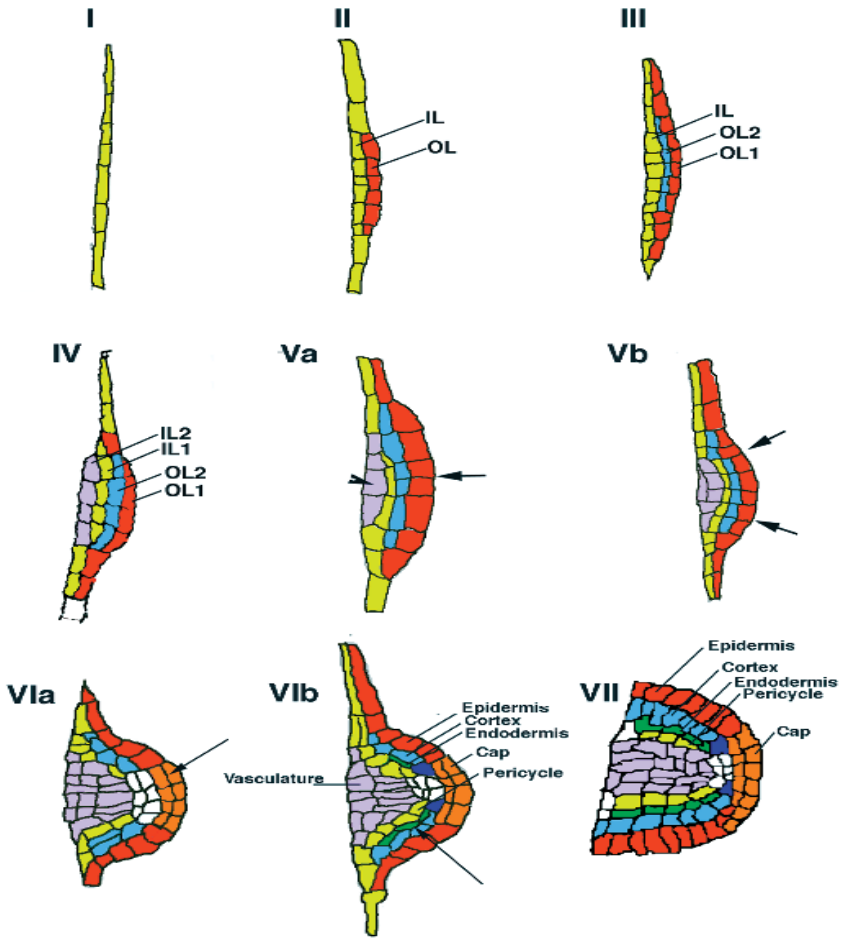


FIG 2. Model of LRP in *Arabidopsis*. Stages are indicated above each diagram. Color coding shows the putative derivation of each tissue from Stage I through Stage VII, based on the information from the histological studies and the marker lines. Note that by Stage VI all the radial pattern elements of the primary root are present in the LRP. The cluster of white cells near the LRP tip at Stages VI and VII cannot be clearly identified, but the position and lack of staining of these cells in differentiated cell-specific marker lines suggest that they develop into initials and quiescent center. White cells at the base of the LRP could not be identified. (Malamy, J. E. and Benfey, P. N. 1997)

The first evidence of the primordia initiation is related to asymmetric anticlinal divisions of the founder cells, in anticlinal cell division the plane of division is at right angles to the surface of the plant body. This way, appear 8 to 10 short pericycle cells, which radially increase their size (Stage I.).

Then continue some periclinal divisions (the plane of division is parallel to the surface of the plant body) producing a two-layered primordium. Outer layer (OL) and inner layer (IL) can be clearly distinguished. Further periclinal division give rise to a three-layered primordium with layers OL1, OL2, IL, and so on a later four-layered primordium with layers OL1, OL2, IL1, IL2 (Stage II.-IV).At this stage in *Arabidopsis* the LRP has penetrated the parent endodermal layer. In some plants including tomato, adjoining endodermis cells, or up to a few layers of the root cortex, divide and contribute to the formation of a primordium (Dubrovsky, J. and Rost, T. L. 2003, Dubrovsky, J. G. et al. 2001, McCully, M. E. 1975; Peterson, R. L. and Peterson, C. A. 1986). Later cells derived from those tissues participate in the formation of a temporary root cap that assists in primordium emergence through the parental root tissues (Barlow, P. W. 2004, Charlton WA. 1996).

In already two-layered stage a forming shape can be distinguished. Not all the small pericycle-derived LRP cells appear to participate in these periclinal divisions; typically the most peripheral cells do divide less frequently. Hence, as the OL and IL cells expand radially the domed shape of the LRP begins to appear. (Ivanchenko, M. G. et al. 2006)

Once primordium have four layers we can talk about an autonomous meristem because studies from Laskowski et al., 1995 concluded, that lateral root formation is a two-stage process divided before and after the developmental point after which an excised LRP could continue to develop in hormone-free media. This functional assay therefore defined the stage (when it has 3-5 cell layers in *Arabidopsis*) at which the LRP is autonomous from the point of view of further development (Laskowski, M. J. et al. 1995). This is just before the cellular architecture of the developing lateral root changes and starts to seem as the radial pattern elements of the primary root. After this just few of the primordia remained arrested, maybe because of some inhibition or failure and do not emerge. Thus, a population of activated cells from earlier stages is a requirement for meristem formation, but is not enough to form a lateral root; two cell layers of primordia don't get to form autonomous meristems when placed in culture (Cheng, J. C. et al. 1995, Laskowski, M. J. et al. 1995).

After this stage a central cell in OL1 and OL2 divides anticlinally to form four small cuboidal cells. The cells adjacent to these two cells in the OL1 and OL2 also divide, creating an outer layer (OL1) that contains 10-12 cells. In addition, cells in IL2 enlarge radially and divide, pushing the overlying layers up and apparently compressing the cells in IL1 and OL2. The LRP at this stage (V.), is midway through the parent cortex(Malamy, J. E. and Benfey, P. N. 1997).

Next, cells of OL2 undergo a periclinal division, creating a new internal layer. These layers are designated OL2a and OL2b. Also, the four central cells of OL1 divide periclinally. By this time, the LRP has passed through the parent cortex layer and has penetrated the epidermis (Stage VI).

In this stage VI, LRP begins to resemble the mature root tip, containing 3 layers that could correspond to epidermis, cortex and endodermis surrounding a core of presumptive stellar tissue, and a potential root cap at the tip of the LRP.

Stage VII. As the primordium enlarges it becomes more difficult to distinguish particular divisions, especially in the internal layers. It appears that many of the cells of the LRP continue to undergo anticlinal divisions. In the OL1, this results in 8-10 cells on either side of 8-10 central cells. Here they used the formula 8-8-8 cell pattern to describe it. The LRP appears to be just emerged from the parent root. Emergence in *Arabidopsis* happens when it is an 8-10-layered primordium

Developmental stages of LRP vary in their structural organizations, once the primordium of the lateral roots emerge from the mother root their organization and structure seems to be very similar(Scheres, B. et al. 1994)

. Now we have technically already a lateral root elongating and not just a primordium. The lateral root in this stage meanly grows by the enlarging of its cells at the base of the root while the outermost cells undergo almost no changes (Malamy, J. E. and Benfey, P. N. 1997).

2.2.2 Emergence of Lateral roots

The development of lateral roots in many species of plants uses to be coupled with temporary structures, which are derived from tissues out of the pericycle. Van Thieghem and Douliot, 1988 said, that this structure origins from the endodermis and some outer laying elements; they called it “Poche digestive”(Van Thieghem, P and Douliot, H. 1888). Von Guttenberg, 1951 limits the origin from this structure only to the endodermis and called it “Tasche”(Schade, Christiane and von Guttenberg, Hermann 1951). In essence, those structures are but the same (Bell, J. K. and McCully, Margaret E. 1970). The function of these structures is considered to be mainly the defence of primordium and the facilitation of emergence (Charlton WA. 1991). In *Convolvulus arvensis* Bonnet, 1969 observed that at first in the surface of primordium was temporary formed “Tasche” structure, which secrete vesicles. These vesicles, evidently originating in dictyosomes, were found in the outermost cells of these structures, and their expected content was hydrolytic enzymes, which implicated dissolution of protoplast and lysis in neighbouring cortical cells(Bonnett, H. T. 1969).

For plants not making these temporary structures, primordia were expected to break out from the parent root only by mechanical force (Charlton, 1991). Bell and McCully, 1970 in the observation of *Zea mays* have concluded that these two methods seem to be possible to combine. In that species, the primordium first creates a temporary "Tasche" structure, which was substituted by mechanical pressure when lignified outer layers of the main root were reached. The activity of hydrolytic enzymes is nowadays expected even in absence of temporary structures (Swarup, K. et al. 2008)The endodermis often divides anticlinally and thus keeps pace with the growth of the primordium, but the other cortical cells are deformed, crushed, pushed aside, and partly degraded by enzymatic activity and other factors (Bell, J. K. and McCully, Margaret E. 1970, Swarup, K. et al. 2008).

Cells in the parent root overlaying new lateral root primordia actively participate in organ emergence thanks to a transcellular auxin signalling network designed to synchronize

lateral root development and emergence processes. The restricted pattern of *LAX3* expression in outer root tissues is important for the localized induction of cell-wall-remodelling genes such as *AIR3*, *PG* and *XTR6(Swarup, K. et al. 2008).*

LAX3 encodes a high affinity auxin influx carrier and facilitates lateral root emergence by promoting the separation of epidermal and cortical cells overlaying primordia. Authors concluded that LAX3 coordinates the spatial expression of several classes of cell-wall-related enzymes, which are likely to act collectively to promote lateral root emergence. IAA3 was only detected in endodermis and is likely to influence the rate of lateral root emergence by regulating the auxin inducible expression of cell-wall-remodelling gene. It was also found that LAX3 expression is auxin inducible and is mediated by the auxin signalling components ARF7, ARF19 and IAA14/SLR(Swarup, K. et al. 2008).

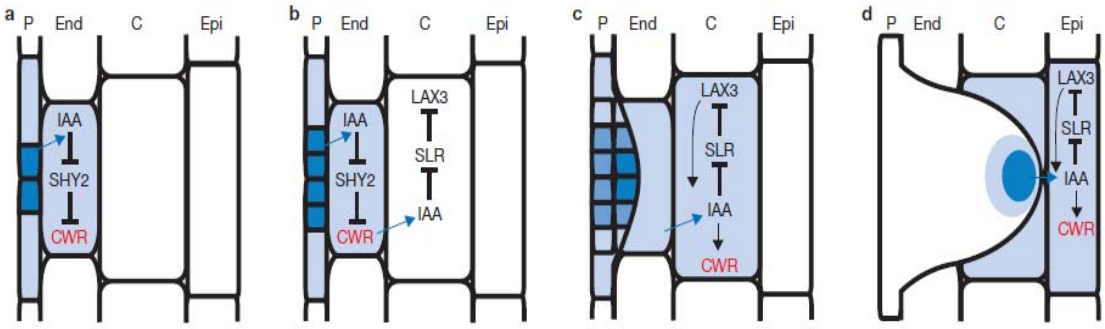


Figure 3 Model for auxin-dependent lateral root emergence. (**a**) Auxin (IAA) originating from dividing pericycle (P) cells induces cell-wall-remodeling (CWR) gene expression in adjacent endodermal (End) cells by targeting the degradation of the SHY2/IAA3 repressor protein. (**b**) Auxin derived from the lateral root primordium also induces expression of the auxin influx carrier LAX3 in adjacent cortical cells (C) by targeting the degradation of the SLR/IAA14 repressor protein. (**c**) LAX3 expression increases cell permeability to auxin, creating a positive-feedback loop. Increased auxin accumulation induces CWR expression. (**d**) At a later stage of primordium development, auxin induces LAX3 expression in adjacent epidermal (Epi) cells. The expression of CWR in a few cells of the different layers facilitates the emergence of lateral root primordium.(Swarup, K. et al. 2008)

Cellular auxin responses are represented as a blue color gradient

(Swarup, K. et al. 2008).

When penetration through parental root tissue occurs, a disturbance of the tissues located outside the pericycle happens, thus creating the possibility of the uncontrolled entry of substances and pathogens that is why this process must be tightly regulated, as cell separation (particularly of the protective epidermal layer) constitutes a risk to the plant internal environment integrity (Nibau, C. et al. 2008). Plants therefore create different structures, such as healing callus like cells, deposition of extracellular material or suberized and lignified cell walls (for cereals) or the accumulation of phenols (in *Hieracium*) resealing perturbed tissues (Peterson R.L. 1979). The purpose of those structures is to restore apoplastic barriers and a continuum of the plant body surface (Charlton, 1991).

LR Development ends with the differentiation of the phloem and xylem in the primordium and their subsequent connection to the phloem and xylem in the parental root. This connection takes place through differentiation of pericycle cells to conductive elements on the proximal end of primordium (Esau, K. 1965, Katherine Esau 1965, Oparka, K. J. et al. 1995).

III. Regulation of lateral root initiation and growth

Physiological processes in plants are surrounded by phytohormones that are the key regulators and coordinators of many functions. Particular response results from their and other factors interaction. Intrinsic regulatory pathways and external factors shape plant body in accord with environmental effects within the limit of its genotype. There is a great diversity of individual tissue or organ responses to phytohormones in contrast with the relative specificity of action of animal hormones. In plants there is a high degree of influence to the reactions of phytohormones by external factors (light, gravity, etc.). Most physiological responses of plants are regulated by two or more participating phytohormones, and this hormone signalling is expected to be involved in both developmental and environmental response pathways (Malamy, J. E. 2005)..

There is very much evidence that some signalling networks are specific for LR formation (Hochholdinger, F. et al. 2004, Rogg, Luise E. et al. 2001) (Coates, J. C. et al. 2006) researchers are now trying to look for novel strategies to manipulate root branching in crop plants (Nibau, C. et al. 2008). And now it is important to move forward to agriculturally relevant plants, especially as the mechanisms at work in crop plants may differ from those in *Arabidopsis (*Nibau*, C. et al. 2008)*

We know, lateral roots in most plants start by growing horizontally, and then eventually turn to grow at or near the vertical. Roots appear to be programmed to grow at a gravitropic set-point angle (GSA) relative to the gravity vector, and this GSA can be genetically perturbed. Hence, GSA can be described as an important, largely overlooked intrinsic component of root system architecture (Malamy, J. E. 2005).

The mechanism responsible for the spatial patterning of LRs and LR primordia remains unknown. However, new convenient measures of LR and LR primordium densities and patterning were developed for Arabidopsis with the use of a protoxylem pericycle-specific enhancer trap line (Dubrovsky, J. G. et al. 2006).

The efficient colonization of soil by plant roots is dependent on the initiation of new lateral roots(Swarup, K. et al. 2008). Most of the lateral root processes are auxin-dependent, or at least are indirectly interacting in complex pathways to control each stage of lateral root development (Casimiro, Ilda et al. 2003).

The main phytohormones involved in lateral root development are auxin and cytokinin, which are drivers of many processes, in some cases acting as antagonists affecting many diverse reactions depending of their concentration, proportion and way of transport. Auxin and other growth regulator are intensively studied long time because of stimulating lateral root formation(Blakely, L. M. et al. 1972). Their suppression by endogenous inhibitors may be responsible for the frequency and distribution of lateral roots on the parent root (Street, H. E and Roberts E.H. 1952).

A integrate system of specific interaction among various phytohormones in diverse steps is up to be discovered. Lateral root ontogeny has been the object of analysis since the end of the nineteenth century (Bell, J. K. and McCully, Margaret E. 1970, Blakely, L. M. et al. 1972, Byrne, J. M. 1973, Van Thieghem, P and Douliot, H. 1888) With the use of new methods this topic is nowadays in an amazingly fast advance. As we see in particular, the availability of nutrients affects both the number and location of lateral root initiation sites (De Smet, Ive et al. 2007, Drew, M. C. 1975, Drew, M. C. and Saker, L. R. 1975, Drew, M. C. and Saker, L. R. 1978, Lloret, P. C. et al. 1998, Lucas, M. et al. 2008). Plants can sense quality of the soil directly via external sensors, and also monitor and respond to their own internal nutrient status. Based on this information, plants must decide whether or not to trigger lateral root initiation. Hence, the formation of lateral roots in the root system provides a good model for studying how plant development is coordinated with environmental conditions(Malamy, J. E. and Ryan, K. S. 2001).

The intrinsic determinants of the root system architecture (RSA) are those which are essential for LR initiation, developmental patterning of the primordium and LR formation and growth.(Malamy, J. E. 2005)

3.1 Developmental processes of LR formation

The developmental processes of LR initiation are different from embryonic root initiation, implying that there are differences in the molecular mechanisms between embryonic root and LR initiation. It has been shown that most of the cells in *Arabidopsis* LR primordia are derived from the central of the three protoxylem pericycle cell files adjacent to the xylem pole (Kurup et al., 2005), suggesting that this central cell file has a specific developmental context for the initiation of LRs.

Although apparently genetically determined, it is not clear why LRs are initiated from the protoxylem pericycle rather than from the protophloem pericycle. In *Arabidopsis*, protophloem pericycle cells are arrested at the G_1 state, but protoxylem pericycle cells are allowed to proceed to G_2 (Beeckman, T. et al. 2001), indicating that protoxylem pericycle cells acquire the competence to divide though located far from the root meristematic zone. Understanding what determines the differential regulation of the cell cycle between the protoxylem and protophloem pericycles is necessary to figure out the LR development. In 2008, Parizot et al. demonstrated, using cytological approaches, that there are two distinct types of pericycle

The protoxylem pole pericycle has meristematic characteristics with frequently three or more vacuoles and a dense cytoplasm containing numerous electron-dense ribosomes. Cells in the pericycle of the phloem pole have a single central vacuole and a parietal cytoplasm with less ribosomes, typical for a more differentiated status (Parizot, Boris et al. 2008).

Interestingly, years before was discovered a very important fact occurring in the pericycle cells cycling, the cell cycle inhibitor Kip-Related Protein2 (KRP2), which blocks G1-S transition, is expressed in the pericycle but excluded from protoxylem pole of pericycle. This supports the idea that, G1- and G2-specific blocks that must each be overcome to achieve lateral root initiation(Malamy, J. E. 2005). At a more distal region, most pericycle cells arrest at G1, but protoxylem pericycle cells proceed to G2 and become

competent for lateral root initiation. This may occur via repression of KRP2 in these cells, which allows cells to proceed beyond G1. In agreement to this concept, over expression of KRP2 leads to a strong reduction in lateral root initiation (Himanen, K. et al. 2002). A subset of the G2-arrested cells then continues on to become founder cells by a still unknown mechanism. Interestingly, KRP2 expression is repressed by auxin (Himanen, K. et al. 2002),consistent with the idea that auxin plays a role in determining which cells become competent for lateral root formation.(Malamy, J. E. 2005)

However, Parizot et al, 2008 determinated that the specification of different pericycle cell types is not controlled by auxin or cytokinin treatment and that their bilateral heterogeneity already occurs in stele initials. Also these two kinds of pericycle cells might have differences in the auxin transport system and/or auxin sensitivity. Analyses with auxin and its inhibitor indicate that both types of pericycle cells react differentially and even in an opposite way to lateral root-inhibiting versus lateral root-inducing conditions.

Differentiated protoxylem element within the xylem is not required for proper pericycle differentiation. To finish, Parizot et al.'s results suggest that pericycle and vasculature determination in the root meristem are controlled by common mechanisms.(Parizot, Boris et al. 2008).

3.2 Phytohormones

3.2.1. Auxins

Auxins plays an important role in many aspects of plant growth amd development, including LR formation, embryogenesis, tropic responces to gravity and light, maintenance of apical dominance, shoot lateral organs initiation and development, vascular formation, and adventitious root formation (Woodward, A. W. and Bartel, B.

2005). Auxin synthesis, conversion between active and inactive forms as well as nonpolar and polar transport take part in the regulatory machinery. In *Arabidopsis*, auxin is transported from young aerial toward the root tip through the root vascular tissues (acropetal transport), and then IAA transported to the root tip is redirected toward the base of the root through the outer cell layers (basipetal transport) (Fig. 1) (according to Morris et al. 2004). Inhibition of such a transport restricts initiation of new LRP. Basipetal polar auxin transport in the root tip also appears to influence LR emergence, despite the fact that LRs emerge relatively far from root tips (Casimiro, Ilda et al. 2001)

Ivanchenko et al., 2006 suggested that cell-cycle activation and lateral root initiation can be partially independent. Protoxylem-adjacent pericycles cells possess the capacity to undergo proliferative cell division that can be related to, and, coordinated with, LR initiation, and that the two processes are apparently auxin-dependent. The dgt mutations uncouple these processes (Ivanchenko, M. G. et al. 2006). The DGT gene encodes a member of cyclophilins, strongly suggesting that the peptidyl-prolyl isomerase activity of DGT may take part in the regulation of auxin transport or in the auxin response necessary for LR formation.(Fukaki, H. and Tasaka, M. 2009).

In addition, genetic studies using *Arabidopsis* mutants demonstrate that an auxin transport system for influx and efflux is necessary for LR initiation and subsequent LR primordium development. Influx is mediated by AUX1 and LAX (Like AUX1), and efflux is mediated by PIN proteins (Benkova, E. et al. 2003, Casimiro, Ilda et al. 2001, De Smet, I. et al. 2006, Fukaki, H. and Tasaka, M. 2009, Nibau, C. et al. 2008).

The bias of IAA transport in plant tissues has been attributed to highly regulated, polarly localized efflux complexes that are characterized by the PIN family of proteins(Friml, J. 2003) PIN proteins characterize auxin efflux carrier complexes, establish the direction of auxin flux, and contribute to localized auxin accumulations that are essential for organ formation and overall plant polarity(Blakeslee, Joshua J. et al. 2005). PIN1 appears to be the primary mediator of IAA movement through vascular tissues to the root tip, with PIN3, PIN4, and PIN7 contributing to this transport in adjacent tissues in the lower root(Blilou, Ikram et al. 2005). Once at the root tip, auxin is redistributed basipetally

through cortical and epidermal cells in a PIN2-dependent transport stream(Benkova, Eva et al. 2003, Blilou, Ikram et al. 2005)In the root elongation zone, PIN2, PIN3, and PIN7 mediate lateral re-direction, or 'reflux', of basipetally transported auxin back into the PIN1-dependent stellar auxin transport stream(Blilou, Ikram et al. 2005, Friml, Jiri et al. 2003). Once re-directed, auxin reaches the root tip and the process is repeated, creating a 'reflux loop' (Blilou, Ikram et al. 2005).

Auxin maxima - 'hot spots' within the root arise as a result of the regulated positioning of auxin transporters within cells, in a process conserved between lateral organ formation in the root and in the shoot (Benkova *et al.*, 2003). Interestingly, auxin signalling regulates the differential positioning of auxin efflux carriers and, consequently, the direction of auxin flow (Sauer, M. et al. 2006). This effect is mediated by the activity of VPS29, a membrane-trafficking component that is involved in the recycling of cargo molecules. Together with other proteins, VPS29 mediates the dynamic arrangement of auxin efflux carriers in response to auxin (Jaillais, Y. et al. 2007). The regulated interplay between auxin transport and signalling is critical for all stages of LR development, and many of the signals regulating RSA impinge upon this pathway(Nibau, C. et al. 2008).

All *Arabidopsis* and cereal mutants affecting auxin production, transport and metabolism have modified LR phenotypes: their involvement in LR formation has been described extensively elsewhere(Casimiro, Ilda et al. 2003, Fukaki, H. et al. 2007, Nibau, C. et al. 2008, Woodward, A. W. and Bartel, B. 2005).

In *Arabidopsis*, the xylem pole pericycle cells, from which LRs arise, are smaller than other pericycle cells, indicating differential cell cycle regulation between pericycle cell types. It is thus suggested that the coordinated action of auxin transport and signalling, cell cycle regulators and novel root-specific proteins is necessary for LR initiation to occur.(Nibau, C. et al. 2008)

Auxin signalling during LR initiation is closely coupled with regulated protein degradation. The process requires an ubiquitin-activating enzyme (E1), an ubiquitin-conjugating enzyme (E2) and an ubiquitin-protein ligase (E3), which transfers ubiquitin

from the E2 to the target ((Petroski MD, Deshaies RJ. 2005). Some E3 ubiquitin ligases consist of multiprotein complexes, and SKP1-CULLIN1-F-box (SCF) E3 ligases contain F-box protein subunits that confer specificity, binding to particular target proteins.

Aux/IAA and ARF proteins may be central to 'reading' auxin concentration gradients and then translating this information into gradients of gene expression that cause morphological and developmental patterning outputs in the plant. Both Aux/IAA and ARF proteins function as transcriptional regulators. While members of the Aux/IAA family are generally thought to act as repressors of auxin-induced gene expression different ARF proteins can either activate or repress transcription. ARF proteins bind to auxin-responsive *cis*-acting promoter elements (AuxREs) using an amino-terminal DNA-binding domain (DBD). It has been hypothesized that the Aux/IAA proteins regulate transcription by modifying ARF activity(Liscum, E. and Reed, J. W. 2002)

Auxin binding to TIR1/AFBs allows them to interact with AUX/IAA proteins and target them for degradation. They dimerize with ARFs, later preventing from binding to promoter elements in auxin responsive genes. Thus aux-induced degradation of AUX/IAA enables ARFs to activate aux-responsive transcription, reviewed in (Nibau, C. et al. 2008).

Lateral roots occurred in a regularly spaced alternating left–right pattern correlating with gravity-induced root waving. Both responses are dependent on the auxin influx transporter, AUX1. Furthermore, auxin responsiveness at the basal meristem oscillates in a periodic manner, correlating with the timing of LR formation. This occurs just under certain conditions (like the inclination of an agar proved in De Smet experiment, etc). Internal space mechanism of LR initiation exists inside the root and this can be shifted by gravitropic responses (De Smet, Ive et al. 2007). Observations of a lateral gradient of auxin responsiveness with a maximum in protoxylem cells made authors believe that auxin accumulation alone is sufficient for priming of founder cells (De Smet, Ive et al. 2007, Nibau, C. et al. 2008). However, many factors have sought to be independent of auxin while regulating LR formation. Lucas et al., 2008 proposed a model of co-regulation between LR formation and gravitropism,, the last concentrate auxin at a certain

point and LR will consume it as in a pool, preventing new LR initiation until the pool had been refilled: this would be accelerated by a new gravitropism (Lucas, M. et al. 2008). Recently, was proved that Local production and subsequent accumulation of auxin in single pericycle cells induced by Cre-Lox-based activation of auxin synthesis converts them into founder cells. Thus, auxin is the local instructive signal that is sufficient for acquisition of founder cell identity and can be considered a morphogenetic trigger in postembryonic plant organogenesis (Dubrovsky, Joseph G. et al. 2008)

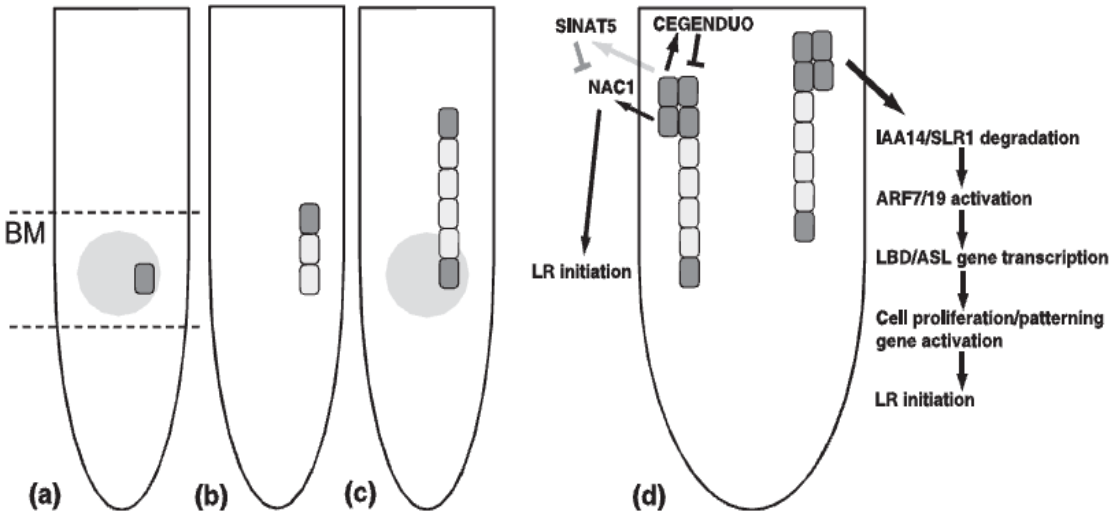


Fig. 4 Aspects of auxin signalling during lateral root (LR) development. (a) A pulse of auxin (light grey) in the basal meristem (BM) primes a pericycle cell (dark grey) to become competent to form a lateral root initial cell. (b) Cells (white) leaving the basal meristem between cyclical auxin maxima is not specified to become LR initials. (c) The first primed pericycle cell arrives at a point where it can initiate LR development; meanwhile another pericycle cell (dark grey) is primed in the basal meristem by the subsequent auxin pulse. (d) Lateral root initiation begins with auxin-induced IAA14 degradation. This allows activation of the ARF7 and ARF19 transcription factors, which activate expression of *LBD*/ *ASL* genes. LBD/ASL proteins in turn activate cell cycle genes and cell patterning genes, enabling formation of a new lateral root primordium (LRP). Auxin also activates transcription of NAC1 to stimulate LR initiation, and at the same time induces expression of two ubiquitin ligases, CEGUENDO and SINAT5, which feed back to attenuate the auxin response(Nibau, C. et al. 2008).

The axr4 (auxin resistant4) mutant has aux1-like phenotypes, including reduced LR formation and reduced root gravitropism(Hobbie, L. and Estelle, M. 1995). While exogenous IAA and 2,4-D, which are transported into the cell by influx carriers, could not rescue the axr4 defects, exogenous NAA, a diffusible synthetic auxin could rescue,

strongly suggesting that AXR4 is involved in auxin influx. In fact, the mutation in the AXR4, encoding a previously unidentified accessory protein of the endoplasmic reticulum (ER), resulted in abnormal accumulation of AUX1 in the ER of root epidermal cells, indicating that AXR4 is required for localization of AUX1 (Dharmasiri, S. et al. 2006).

In the gain-of-function solitary root (slr) mutant, which carries a stabilizing mutation in the IAA14 negative regulator of auxin signalling, shows deficient early LR primordium formation because cell division is not maintained in the pericycle (Fukaki, H. et al. 2002). Similarly, the gain-of-function mutant massugu2 (msg2), defective in IAA19, displays a significantly decreased LR number. IAA19 inhibits the activity of the auxin response factor (ARF) NON-PHOTOTROPIC HYPOCOTYL 4 (NPH4)/ARF7, a positive regulator of LR formation (Osmont, Karen S. et al. 2007b, Tatematsu, K. et al. 2004).

In wild-type plants, auxin triggers the degradation of IAA14, enabling ARF7 and ARF19 to activate transcription of *LATERAL ORGAN BOUNDARIES DOMAIN*/*ASYMMETRIC LEAVES LIKE* (*LBD*/*ASL*) genes (Okushima, Y. et al. 2005). ARF7 also interacts with a MYB transcription factor that provides a link among auxin, LR initiation and environmental responses (Shin, R. et al. 2007). IAA28 is also important for LR initiation. The gain-of function mutant *iaa28* forms fewer LRs than the wild type: IAA28 is degraded by auxin and represses auxin-induced LR-formation genes. However, *IAA28* mRNA levels are repressed by auxin, indicating a complex regulation of *IAA28* during auxin signalling ((Dreher, K. A. et al. 2006, Rogg, Luise E. et al. 2001). The *iaa28* mutant is also resistant to exogenous cytokinins and ethylene, suggesting an integration point for other hormone pathways.(Nibau, C. et al. 2008) In contrast, *iaa28-1* roots display normal sensitivity to the phytohormones abscisic acid, methyl jasmonate, and epibrassinolide, and *iaa28-1* hypocotyls respond normally to aminocyclocarboxylic acid over a range of concentrations(Rogg, Luise E. et al. 2001).

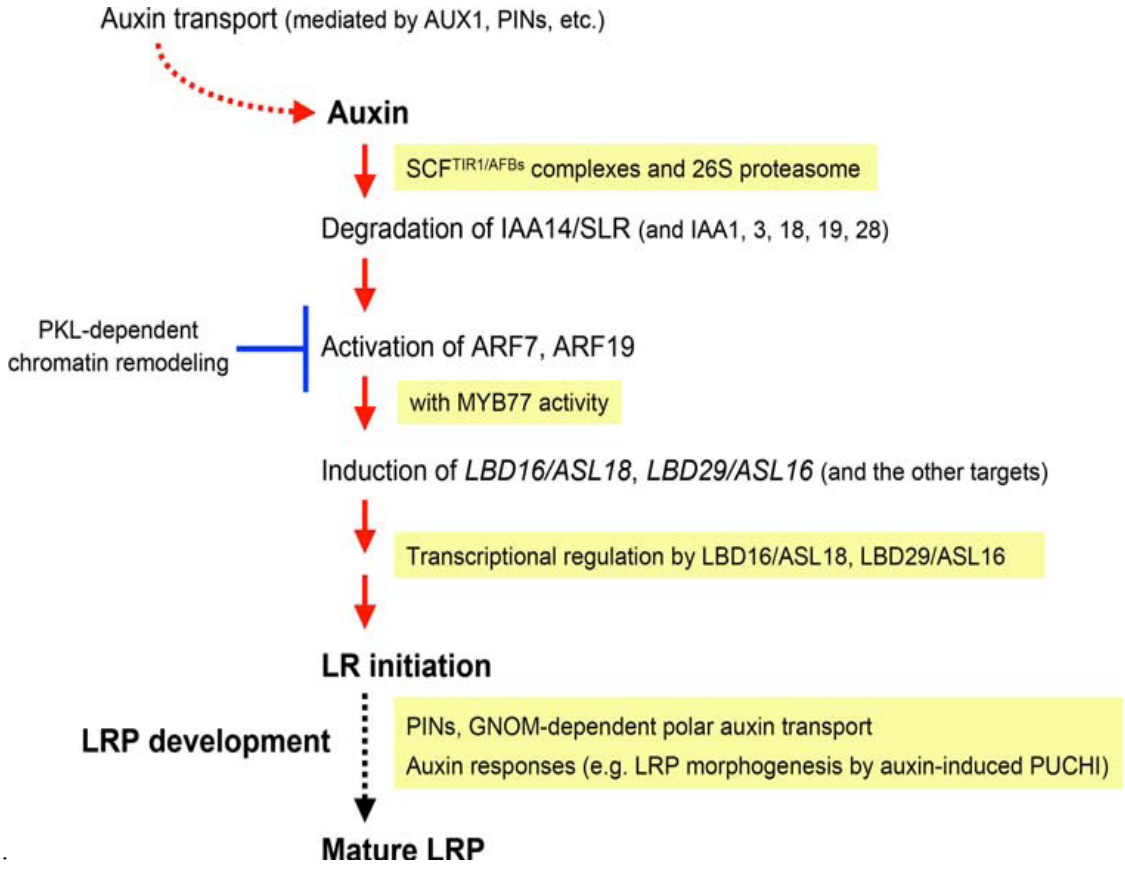


Fig. 5. Auxin signaling pathway model for LR initition and LR primordium development. Auxin signals that are transported to the protoxylem pericycle cells (mediated by AUX1, PINs, etc.) promote degradation of the Aux/IAAs (IAA14/SLR etc.) involved in LR initiation through SCFTIR1/AFBs complexes and 26S proteasome, resulting in the activation of ARF7/ARF19 function, and allowing ARF7/ARF19 to activate the target genes required for LR initiation (LBD16/ASL18, LBD29/ASL16, and the other targets). LBD16/ASL18 and LBD29/ASL16 are nuclear proteins that are also involved in transcriptional regulation for LR initiation. This results in anticlinal cell divisions in the protoxylem pericycle for LR initiation. Subsequent LR primordium (LRP) development occurs through PINs, GNOM-dependent polar auxin transport, and morphogenesis mediated by auxin-induced PUCHI signaling. PKL-dependent chromatin remodeling is required for IAA14/SLR-mediated inactivation of ARF7/ARF19 activity during LR initiation(Fukaki, H. and Tasaka, M. 2009)

Auxin effect on LR formation is mediated by VIER F-BOX PROTEINE (VFB) F-box proteins in *Arabidopsis*. VFBs may regulate auxin-induced gene expression, and consequently LR formation, by a pathway independent of the auxin receptor TIR1 (Schwager, K. M. et al. 2007). Interestingly, auxin stimulates the transcription of

ubiquitin ligases that repress auxin signals, providing an elegant feedback mechanism to maintain auxin sensitivity in the pericycle. The F-box protein CEGENDUO (CEG) is a negative regulator of LR formation whose transcription is induced by auxin(Dong, L. et al. 2006). Auxin in lateral root interaction is highly dependent of the ubiquitin-proteasome pathway, both to transduce signals by degrading repressors and also to reset the system by destroying activators when they are no longer needed. Protein degradation allows for rapid changes in response to the ever-changing environment, as well as providing fine-tuning to sustained signals.

As indicated above auxin acts as a central point of LR development. There are various interactions of auxin with other regulatory elements including other phytohormones. One of such known relationships is between auxin and ethylene.

3.2.2 Ethylene

Expression of ACC synthase, a rate-limiting enzyme for ethylene biosynthesis, is strongly auxin inducible (Swarup, Ranjan et al. 2002). ACC inhibits both the initiation and the elongation of lateral roots and can be reversed by auxin.(Negi, S. et al. 2008) New works agree in that auxin not only influences ethylene homeostasis, but also vice versa (Nibau, C. et al. 2008, Stepanova, Anna N. et al. 2007). The mechanism for the IAA and ethylene antagonism during lateral root formation is more complex to dissect than the synergistic action of these two hormones on root elongation. As AUX1 is expressed in the root tip, in developing lateral roots and in the shoot meristem(Marchant, A. et al. 2002), it is not possible to determine which of these sites control the movement of auxin needed to inhibit lateral root formation in response to ethylene. The reduced acropetal transport in aux1 may be caused by the altered loading of auxin from the leaves and cotyledons (Marchant et al., 2002) into the acropetal IAA transport stream. Even

though the role of AUX1 in mediating IAA transport is complex, AUX1 is clearly tied to ethylene-regulated root development.(Negi, S. et al. 2008)

We are learning more about other phytohormones as salicylic acid, which promotes LR initiation, emergence and growth, possibly via crosstalk with cytokinin or auxin (Echevarria-Machado, I. et al. 2007). Many auxin independent signalling pathways regulating lateral root formation are known; as example ARABIDILLO-1 and -2 which may form ubiquitin E3 ligases (Coates, J. C. et al. 2006) or the *alf4* mutant (ABERRANT LATERAL ROOT FORMATION 4) a mutant case showing a complete absence of LRs which maintains full responsiveness to auxin inhibition of primary root elongation (DiDonato, R. J. et al. 2004).

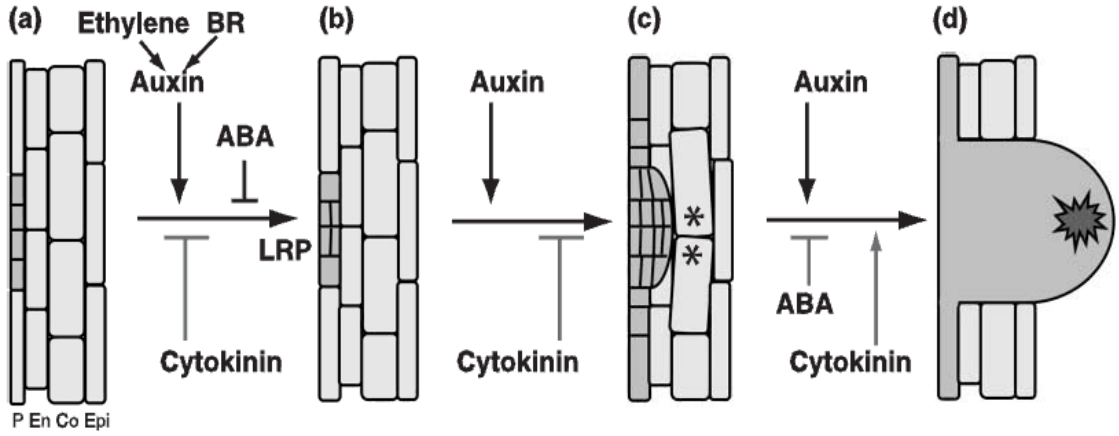


Fig. 6 Lateral root development in *Arabidopsis* shown in longitudinal section. P, pericycle; En, endodermis; Co, cortex; Epi, epidermis. (a) Early initiation – a founder xylem pole pericycle cell (dark grey) undergoes initial anticlinal cell divisions (perpendicular to the surface of the root). (b) Periclinal cell divisions (parallel to the surface of the root) begin and the lateral root primordium (LRP) begins to grow. (c) The LRP undergoes further organized cell divisions and begins to emerge through the outer cell layers of the primary root, resulting in cell separation (asterisks). (d) The new lateral root is fully emerged and its new meristem is activated (dark grey star). It will continue to grow and elongate. At each stage, the effect of various key plant hormones is indicated. ABA, abscisic acid; BR, Brassinosteroids(Nibau, C. et al. 2008).

3.2.3 Cytokinins

Recent studies have indicated that CK is an endogenous negative regulator of LR formation(Fukaki, H. and Tasaka, M. 2009).

Many reports describe the inhibitory effect of cytokinins on lateral root formation. In opposite reduced CK levels increases LR numbers. CK deficiency was also associated with unusually close spacing of LR primordia (Malamy, J. E. 2005, Werner, T. et al. 2003). Results obtained using an ethylene-insensitive mutant and an inhibitor of ethylene biosynthesis indicate that cytokinins exert their effects on lateral root development independently of ethylene (Laplaze, Laurent et al. 2007).The negative CK effect on the number of cells within the cell division zone in primary root meristems is ethylene-independent (Kuderova, Alena et al. 2008). In spite that the inhibition of primary root growth by cytokinins appears to be ethylene dependent (Laplaze, Laurent et al. 2007).

Cytokinins appear to perturb the formation of auxin maximum required for LRP formation from first division on (Li, X. et al. 2006). Laplaze et al., 2007 and Kuderova 2008, suggest that cytokinins inhibit lateral root initiation by interfering either directly or indirectly with PIN-dependent auxin distribution. They influence lateral root formation independently of ethylene at a very early developmental stage, it seems that they interfere with the initial asymmetric division; the early establishment of an auxin gradient is affected, too. The authors' results indicate that cytokinins do not block lateral root initiation in lateral root founder cells by acting on auxin-induced cell division but rather by inhibiting auxin-induced cell fate respecification by downregulating PIN gene expression in *Arabidopsis thaliana* and thus, its transport in a complex network regulating the root architecture.

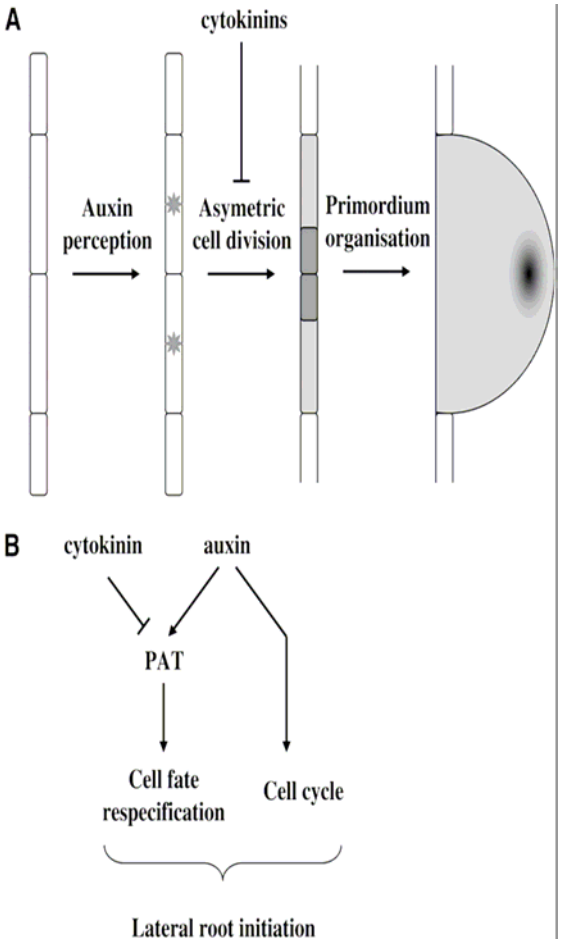


Figure 7. Model of Cytokinin–Auxin Interaction during Root Development. (A) Lateral root initiation is triggered by auxin perception by some xylem pole pericycle cells leading to an asymmetric cell division. Cytokinins do not block auxin perception or response in lateral root founder cells but act downstream to perturb the asymmetric cell division. (B) Lateral root initiation requires auxin-induced cell fate respecification and cell cycle progression (Vanneste, Steffen et al. 2005). Cell fate respecification depends on the expression of PIN genes to create an auxin gradient responsible for asymmetric cell division and acquisition of LRP identity. Cytokinins inhibit this step by downregulating PIN gene expression (Laplaze, Laurent et al. 2007)

Experiments with lowered endogenous and increased exogenous CK levels (lo Ioio, R. et al. 2007, Werner, T. et al. 2003), respectively) have demonstrated that CK controls the root elongation primarily by controlling the size of the meristem rather than by changes in cell division rate. Kuderová et al., 2008 concluded that the increase in endogenous CKs affects root elongation by reducing the number of dividing cells in the meristem. Their experiment demonstrates that in response to the endogenous CK overproduction, the LRPs´ growth is inhibited during developmental stages II-IV and their transition to stage V (Kuderova, Alena et al. 2008). After finding that a negative role for CK in the regulation of the size of root meristems (Werner et al., 2003) and that disruption of CK signalling results in reduction of the root meristem size, too, (Ferreira, F. J. and Kieber, J. J. 2005) came up with the hypothesis which explains the phenomenon by what is called

supraoptimal endogenous CK levels in the root, and that later agreed with the observation of Kunderova et al, 2008 where meristem reduction results from the ipt-dependent CK enhancement. The CK biosynthesis is realized in LR meristems thanks to the development-specific expression of the AtIPT genes, AtIPT5 is predominantly expressed in the LRPs (Miyawaki, K. et al. 2004) Checking in CK contents, reducing it within a physiological limits to optimal levels (e.g. by CKX activity) would lead to an acceleration of root growth, while a complete inhibition of CK signalling would result in reduction of primary root growth, what correlates with a reduction in meristem size. Similarly, overdose of the root with CK would lead to meristem reduction, too.

CK delimits the size of the root meristem by induction of cell differentiation at the transition zone (Ioio et al. 2007). Thus, temporal a spatial specificity of CK action must be taken into account when studying the complex regulation of formation and maintenance of the root meristems.

Effects of enhanced CK levels on auxin distribution and the development of LRPs

CKs may participate in regulating LR development at different stages through the regulation of distinct CK-dependent signalling pathways that might reflect both essential developmental programs and other environmental stimuli and adaptive responses. Endogenous and exogenous CK levels seem to be monitored by distinct *A. thaliana* histidine kinases (AHKs)(Kuderova, Alena et al. 2008).

Lopez-Bucio et al, 2007 worked on alkamides, lipid-based metabolites that may regulate meristematic activity and pericycle cell activation (Lopez-Bucio, J. et al. 2007). This regulatory mechanism seems to involve the cytokinin receptors.

Li et al. (2006) demonstrated that CK treatment inhibits LR initiation by blocking pericycle founder cell cycling during the G2 to M transition phase. Surprisingly, exogenously applied auxin cannot rescue the CK-mediated inhibition of LR initiation per se but it can rescue cell divisions (Laplaze, Laurent et al. 2007, Li, X. et al. 2006). This indicates that CK accumulation in pericycle cells does not prevent the auxin-mediated

activation of cell divisions but blocks the developmental program of LR initiation(Fukaki, H. and Tasaka, M. 2009).

LRP initiation seems to depend on the primary root basipetal auxin transport (Casimiro, I. et al. 2001, De Smet, Ive et al. 2007), while their emergence is positively influenced by the acropetal auxin transport via the phloem(Casimiro, I. et al. 2001). New data imply possible interference of CK with polar cell-to-cell auxin transport within developing LRPs. But no significant effect in the maintenance of auxin distribution responsible for stem cell organization or any obvious changes in the cell division pattern in the main root meristem of seedlings is exhibiting the intermediate phenotype (Kuderova, Alena et al. 2008)

Similar to *Arabidopsis*, the legume *Medicago truncatula* also uses CK signalling to inhibit LR formation. RNA interference of the CK receptor homolog Cytokinin Response1 (MtCRE1) led to CK-insensitive roots, which had an increased number of LRs(Gonzalez-Rizzo, S. et al. 2006), indicating a common roles for CK in higher plant LR formation(Reviewed in Fukaki, H. and Tasaka, M. 2009)

The fact that, the responsiveness of a 3-day-old plant is greater to the transient increase of the endogenous CK compared with a 6-day-old plant implies a possible role for CK during primary meristem maturation together with the existence of mechanism to fine-tune the CK level and auxin temporally at specific stages of root development. This suggests the presence of development-specific mechanisms involved in regulation of CK metabolism and/or signalling during the first 6d of A. thaliana development. Differences in the specificity of inactivation of CKs by glucosylation might be one oh the factors responsible for the development-specific sensitivity of the immature root meristems in A. Thaliana (Kuderova, Alena et al. 2008). The molecular mechanisms that control CK glycosylation and regulate the complex net of CK metabolism and signalling might act differently in early and late stages and further work has to be done to fully understand them.

3.2.4 Gibberilins

Gibberellic acid (GA) biosynthesis has been detected in root tips of different plants and GA signalling is indeed required for primary root growth (Fu, X. D. and Harberd, N. P. 2003, Kaneko, M. et al. 2003, Osmont, Karen S. et al. 2007b). However, a major role for GA in root branching has never been clearly demonstrated, although GA acts synergistically together with ethylene to promote both initiation and growth of adventitious roots in flooded rice plants (Steffens, B. et al. 2006). *Arabidopsis* GA deficient mutants have reduced primary root growth(Fu, X. D. and Harberd, N. P. 2003). Moreover is known now that GA helps to atenuate low Pi responses(Jiang, Caifu et al. 2007) and takes part in regulation of nodules formation on pea roots (Ferguson, B. J. et al. 2005)

3.2.5 ABA

Abscisic acid (ABA) regulates many aspects of plant growth and development, yet many ABA response mutants present only subtle phenotypic defects, especially in the absence of stress. By contrast, the ABA-insensitive8 (abi8) mutant, isolated on the basis of ABA-resistant germination, also displays severely stunted growth, defective stomatal regulation, altered ABA-responsive gene expression, delayed flowering, and male sterility. The stunted growth of the mutant is not rescued by gibberellin, brassinosteroid, or indole-acetic acid application and is not attributable to excessive ethylene response, but supplementing the medium with Glc improves viability and root growth. It was found that the ABI8-GUS fusion protein expressed under control of its own promoter is localized primarily to the elongation zone of roots, where it accumulates in a punctuate pattern within the cytoplasm (Brocard-Gifford, I. et al. 2004).

The involvement of abscisic acid (ABA) in LR formation has been summarized mainly from exogenous application and ABA related signalling mutants in *Arabidopsis* (Fukaki, H. and Tasaka, M. 2009) Exogenous ABA inhibits LRP emergence prior to activation of the LR meristem, and exogenous auxin is not able to rescue it, indicating that an ABA sensitive, auxin-independent checkpoint is involved at the post-emergence stage (De Smet, I. et al. 2003)There is also genetic evidence for ABA-auxin regulatory interaction in LR formation. The ABI3 (ABA INSENSITIVE3) gene, encoding a B3 type transcription factor necessary for ABA signalling, is auxin-inducible in LR primordia (Brady, S. M. et al. 2003). Mutations in ABI3 attenuate the responsiveness of LR formation to exogenous auxin or auxin transport inhibitor. In contrast, mutations in ERA1 (ENHANCED RESPONSE TO ABA1), which encode a farnesyl transferase, increase the number of LRs. Therefore, while exogenous ABA negatively regulates LR emergence as mentioned above, ABA signaling mediated by ERA1 and ABI3 is necessary for auxin-mediated LR formation (Fukaki, H. and Tasaka, M. 2009).

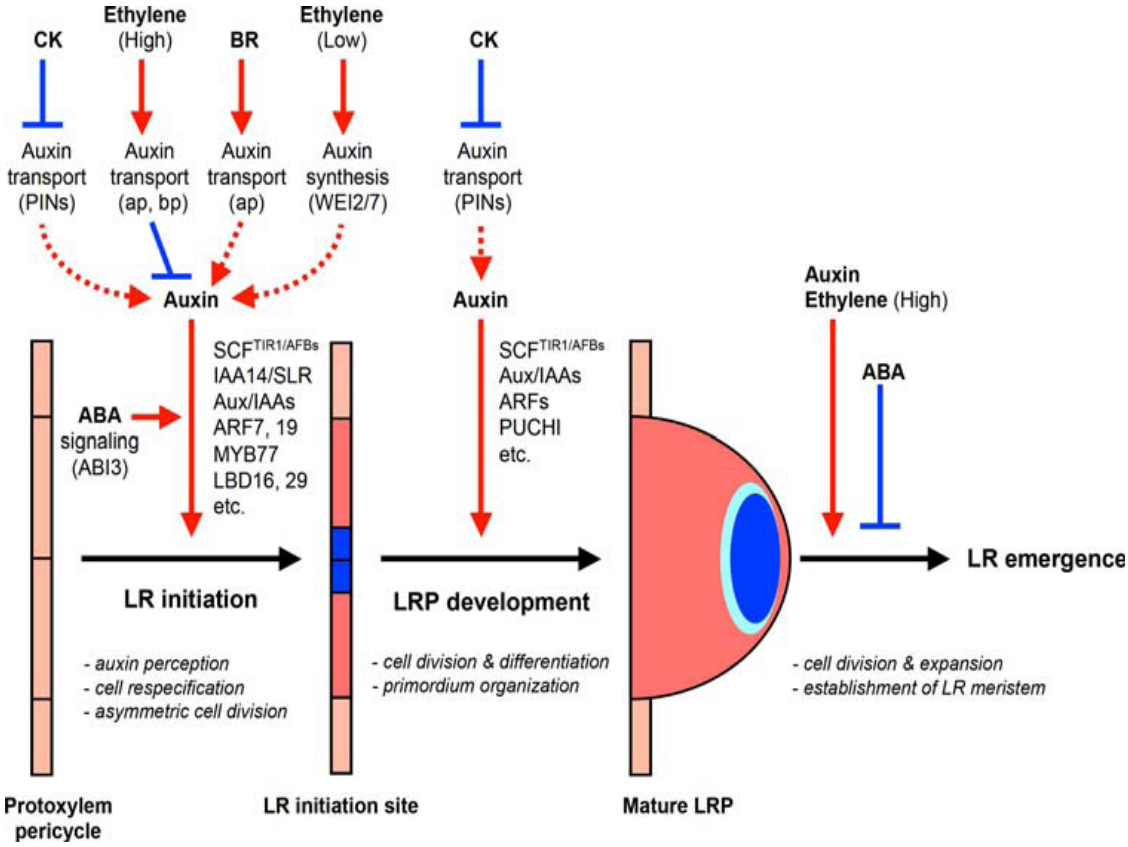


Fig. 8. Hormone interactions during LR formation. LR initiation is positively regulated by auxin but negatively regulated by CK and high concentrations of ethylene (high concentrations of exogenous ACC). The polar auxin transport with a balance of influx and efflux in both acropetal and basipetal directions is necessary for LR initiation and setting up auxin gradient to organize LR primordium (LRP) (blue color in LR initiation site and primordium). CK inhibits auxin maxima by altering the expression of PINs, thereby inhibiting auxin gradient for LR initiation. High concentrations of ethylene (high concentrations of exogenous ACC) enhanced both acropetal (ap) and basipetal (bp) auxin transport, inhibiting LR initiation. BR promotes LR initiation by increasing acropetal (ap) auxin transport. Low concentrations of ethylene (low concentrations of exogenous ACC) promote LR initiation by increasing Trp-dependent auxin synthesis mediated by WEI2 and WEI7. Normal ABA signaling mediated by ABI3 is necessary for proper auxin responsiveness for LR initiation. Auxin also promotes LR primordium development but CK inhibits LR primordium development and affects auxin maxima by altering the expression of PINs. ABA inhibits LR emergence whereas auxin and ethylene (via high concentrations of exogenous ACC) promotes LR emergence. Red arrows and blue bars indicate positive and negative regulation during LR formation, respectively(Fukaki, H. and Tasaka, M. 2009)

All of the ABA response mutants in *Arabidopsis* are morphologically similar, in that they are sterile and have severely stunted growth because of reduced cell elongation that is not rescued by treatment with any known hormones or inhibitors of hormone synthesis/response Microscopic examination of abi8 roots and hypocotyls showed that the improved root growth on Glc reflected maintenance of the root apical meristem and improved vascular development but that the mutant cells still were much shorter than those of the wild type. These results suggest that the cellulose synthesis defect still inhibits cell elongation, but tissue differentiation and overall morphology are subject to additional regulation. abi8 growth is not only dependent on low concentrations of Glc but also is resistant to the inhibitory effects of high Glc, suggesting a defect in sugar signalling and/or transport. The related enzymes are generally encoded by multigene families, with specific family members exhibiting opposite responses to sugars, such that some are induced by feast and others by famine conditions (Koch, K. E. 1996)(Koch, 1996), and it is not clear which of the A. thaliana genes are regulated by Glc and/or ABA. ABA might be involved in preventing production of hydrolases needed for emergence and/or reactivation of cell cycling in postgerminative growth. (Brocard-Gifford, I. et al. 2004). This author explains that the abi8/eld1/kob1 (similar phenotypes) mutants provide evidence linking ABA and/or Glc signalling to promotion of cellulose biosynthesis and organizing vascular differentiation and provide an opportunity to decipher the function of a novel essential protein and possibly a novel signalling mechanism.

(Monroe-Augustus, Melanie et al. 2003) isolated *ibr5* as an *Arabidopsis* indole-3-butyric acid–response mutant, but it also is less responsive to indole-3-acetic acid, synthetic auxins, auxin transport inhibitors, and the phytohormone abscisic acid. *IBR5* encodes a 257– amino acid protein with 35% identity to known dual-specificity mitogen-activated protein kinase (MAPK) phosphatases. Dual-specificity phosphatases often dephosphorylate signalling components; therefore, IBR5 may modulate auxin and ABA signal transduction pathways(Monroe-Augustus, Melanie et al. 2003), and promotes

auxin responses through a novel mechanism distinct from TIR1-mediated Aux/IAA repressor degradation (Strader, L. C. et al. 2008).

Because *ibr5* has decreased sensitivity to auxin and ABA and is defective in an apparent dual-specificity phosphatase, we can envision several possible roles for IBR5. One possibility is that IBR5 dephosphorylates a single MAPK acting in a signalling pathway. Because MAPK phosphatases inactivate MAPKs and the loss-of-function *ibr5* mutants are less responsive to auxin and ABA, this putative MAPK may normally inhibit both auxin and ABA responses. The integration of auxin and ABA responses could be either a direct or an indirect result of this signalling. It also is possible that IBR5 has more than one MAPK substrate. IBR5 may dephosphorylate a protein or proteins not involved in a canonical signalling pathway, and the loss of this dephosphorylation reduces sensitivity to auxin and ABA (Monroe-Augustus, Melanie et al. 2003). On the other hand, a gain-of function mutant in Aux/IAAs, axr2-1/iaa7 is resistant to exogenous ABA whereas the slr-1/iaa14 mutant is hypersensitive to ABA in primary root growth inhibition assays; Fukaki et al. 2002), suggesting that Aux/IAA-dependent auxin signalling also affects ABA activity in the roots. Therefore, there may be several types of interactions between ABA and auxin in auxin-mediated root growth and development(Fukaki, H. and Tasaka, M. 2009).

The inhibitory effect of NO_3^- is significantly reduced in several ABA insensitive mutants (abi4 and abi5) and ABA synthesis mutants (aba1, aba2 and aba3) but not in other ABA insensitive mutants (abi1, abi2 and abi3). This indicates that the inhibitory effect of NO_3^- on LR development involves a specific ABA signal transduction pathway mediated by ABI4 and ABI5 in *Arabidopsis* roots(Fukaki, H. and Tasaka, M. 2009).

3.2.5 Brassinosteroids

Auxin is crucial for lateral root development and lateral root emergence can be blocked by the auxin transport inhibitor N-(1-naphthyl) phthalamic acid (NPA; Reed et al., 1998; Casimiro et al., 2001). 2 μM NPA not only inhibited lateral root formation in wild-type seedlings but also reduced brassinolide (BL) promotion of lateral root formation (Fig.1B). These results imply a potential interaction between BRs and auxin in the promotion of lateral root formation, i.e. BRs may act through auxin to activate lateral root development(Bao, Fang et al. 2004).

GUS expression pattern in DR5::GUS transgenic seedlings facilitate the examination of different stages of LRP, as DR5::GUS is expressed at all stages of LRP (Benkova´ et al., 2003) and thus makes easier the identification of early LRP (Bao, Fang et al. 2004). They found interesting that NPA almost completely suppressed BL induced increase in the number of stage 1 LRP and that 2 μM NPA dramatically decreased the number of stage 2 to 7 LRP but slightly increased the number of stage 1 LRP. BRs regulate auxin transport, providing a novel mechanism for hormonal interactions in plants and supporting the hypothesis that BRs promote lateral root development by increasing acropetal auxin transport(do not affect the overall IAA level of whole seedlings) (Bao, Fang et al. 2004). Furthermore, these authors said that it remains possible that BR-auxin cross-talk involves auxin action through its regulation of BR accumulation or sensitivity, and many questions remain about the mechanisms by which BRs interact with auxin.

In rice, a casein kinase 1 gene, OsCKI, is upregulated by both brassinosteroid and abscisic acid (ABA) and promotes lateral and adventitious root formation as well as cell elongation. OsCKI may affect LR development by regulating endogenous auxin levels (Liu, W. et al. 2003, Malamy, J. E. 2005).

3.3 Modulation of root system architecture by abiotic factors:

N, P, S, K and Fe as signals for the control of root development.

The production of nutrient-rich crops is one of major aims of biotechnology. Iron deficiency is the most common human nutritional disorder in the world. In plant, Iron is an important micronutrient, it is an essential cofactor for two important biological processes, photosynthesis and nitrogen fixation, and the availability of iron greatly influences plant physiology and morphology (Hong-Qing Ling et al. 2002) New studies showed that the formation of root hairs in response to iron deficiency is associated with cell-specific accumulation of transcripts that are involved in iron acquisition(Santi, Simonetta and Schmidt, Wolfgang 2008).

One very important element, Nitrogen is one of the most abundant elements on earth. However, It is also commonly the most limiting elements for plant growth because e of its low availability in the soil and because nitrate, the most common form of N fertilizer, is highly soluble in the soil solution and can be easily lost by leaching or by bacterial denitrification (CP Vance 2001). P is the second most critical factor in determining plant productivity because the anionic form of phosphate, in which P is assimilated by plants , is extremely insoluble in the soil solution.(Lopez-Bucio, J. et al. 2002). Changes in nitrate and phosphate availability have contrasting effects on lateral root formation and elongation (Zhang, Hanma and Forde, Brian G. 1998).Global availability and localised supply have different effects on the root system. Not just in *Arabidopsis*, increasing global nitrate availability reduces primary root elongation, whereas an increase in P supply helps primary root elongation but the density of lateral root decreases dramatically while increasing nitrate does almost no effect across a range. Lateral root elongation by both high nitrate and high phosphate availability is suppressed (Drew, M. C. and Saker, L. R. 1975, Lopez, B. C. et al. 2003).

In plants grown on a low nitrate concentration (10 μM), exposure of a section of the primary root to high nitrate induces a local stimulation of lateral root elongation whereas, uniformly, high nitrate (10 mM) reduces lateral root elongation throughout the root system(Zhang, Hanma et al. 1999). The global inhibitory effect of nitrate seems to be a response to a nitrate sufficiency status because lateral root elongation under these conditions is inhibited even in regions of the root system that are growing in low nitrate concentrations. This is caused by the signalling effect of nitrate itself rather than being a response to downstream metabolites (Zhang, Hanma and Forde, Brian G. 1998). So, NO_3^- signal can have two effects on LR, a localized stimulatory effect, which is mediated by 2 mediators, first, ANR1, a NO_3^- locally inducible Arabidopsis gene to local nitrate response, that encodes a NITRATE REGULATED-1 MADS-box transcription factor (A MADS box is one of the well known homeotic genes), Second a systemic inhibitory effect of high internal nitrate status. Auxin might mediate localized responses to N, whereas ABA mediates systemic responses (Osmont, Karen S. et al. 2007a) Seven other MADS box genes have a similar expression pattern to ANR1 under different nitrate conditions (Gan, Y. B. et al. 2005). Metabolic mechanisms are not involved in the control of the *Arabidopsis* root system to nitrate and so nitrate is perceived as a signalling molecule.

Carnitine, an organic nitrogenous cation, induces LR formation. However , disruption of an *Arabidopsis* plasma membrane-localized carnitine transporter, AtPCT1, led to increased root branching, local concentration of carnitine in the root may affect the C:N ratio and hence LR development(Lelandais-Briere, C. et al. 2007).

Axr4, an auxin-response mutant can not respond to localized nitrate supplies, showing an overlap between the auxin and nitrate responses (Zhang, Hanma et al. 1999). It was also found that when *Arabidopsis* plants are grown on high ratios of sucrose to nitrogen, lateral root initiation is almost completely inhibited, apparently by accumulation of auxin in the hypocotyl, assessed by activity of the DR5 promoter. Hence, it is tempting to speculate that lateral root initiation is blocked under these conditions by preventing auxin

movement from the shoot system to the root system (Malamy, J. E. and Ryan, K. S. 2001).

Some species, including barley and cedar, but not *Arabidopsis* are also able to respond to a localized ammonium supply (Drew, M. C. 1975, Nibau, C. et al. 2008, Zhang, Hanma et al. 1999). Local applications of NO_3^- and NO_4^+ at a low or a high concentration had local effects on elongation and branching of the root system. Contrasting effects of ammonium and nitrate were observed on the apical diameter of tap-roots and lateral roots (Boukcim, H. et al. 2006).

Van der Weele *et al*. (2000) used PEG to simulate water stress, and found that severe water stress reduced lateral root numbers, potentially through reduced lateral root initiation.(van der Weele, Corine M. et al. 2000). He found that the effects of nitrate in nutrient media can be reproduced with the addition of mannitol (increased concentrations of osmotica).

ABA-deficient mutants *aba2* and *aba3* were less sensitive to mannitol repression, indicating a critical role for ABA in this process. This is interesting because primordia in the root system develop slowly when water is limited, perhaps due to the increases in ABA that are associated with many osmotic responses. The repressed primordia can then be rapidly activated when water becomes available for uptake (Malamy, J. E. 2005) Thanks to dig3 mutant, drought inhibition of lateral root growth, authors thinks that ABA and drought response involves factors required more generally for growth(Nibau, C. et al. 2008, Xiong, L. M. et al. 2006).

ABA plays a central role in mediating the inhibitory effect of NO_3^- on lateral root elongation (Signora, L. et al. 2001). In *lotus japonicus*, the CLAVATA1 homolog, HAR1 is required for shoot-controlled regulation of grow and the nitrate sensivity of symbiotic development. HAR1 (HYPERNODULATION ABERRANT ROOT1) locus have a hypernodulation phenotype in the presence of *Rhizobium* and increased lateral root formation in absence of this symbiotic bacterium. This is a yet unknown mechanism that

integrates root and shoot signalling still not found in *Arabidopsis* (Krusell, Lene et al. 2002, Nishimura, Rieko et al. 2002).

Phosphate is a common limiting factor of many infertile soils. A legume, white lupin (*Lupinus albus L.*) is well established in this type of low P availability soils, because when P is insufficient, white lupin forms proteoid roots, which are clusters of short lateral roots that arise from the pericycle and are specialized in P uptake (Johnson, J. F. et al. 1996). They secrete organic acids and phosphatases into the surrounding soul to solubilize phosphate and aid its uptake (Nibau, C. et al. 2008, Schulze, J. et al. 2006). When growing *Arabidopsis* in such conditions, its architecture resembles that of the proteoid roots of lupins(Lopez, B. C. et al. 2003).

P also acts as a signalling molecule, locally stimulating LR elongation or as an inhibitory effect when growing on high external concentrations. Studies suggest that this responses may be ubiquitous in the plant kingdom (Williamson, L. C. et al. 2001). Authors analyses have shown that low-P-grown mature roots lack a normal apex and have increased expression of P transporter genes, by contrast, the root of high-P-grown plants have high auxin concentrations in their meristems and their cells have high mitotic activity, which correlates with their reduced expression of genes that encode high- affinity P transporters(Lopez, B. C. et al. 2003). Interestingly, many root responses to phosphate starvation are repressed by cytokinin signalling (Franco-Zorrilla, Jose Manuel et al. 2005). In addition, Pi starvation affects gibberellins signalling in roots, whereas gibberellin can attenuate the low-Pi response (Jiang, Caifu et al. 2007, Nibau, C. et al. 2008)).Under limiting (1µM) P concentration lupins and *Arabidopsis* are more sensitive to auxins in terms of the inhibition of primary root elongation and increase of lateral root density, but also cytokinins suppress lateral root initiation.(Lopez-Bucio, J. et al. 2002). Cytokinins repress the expression of low-P-regulated genes, suggesting that these hormones not just control the architecture but other aspects of the low-P rescue response(Lopez, B. C. et al. 2003).

A *phosphate deficiency response* (*pdr*) mutant that is hypersensitive to low phosphate was isolated (Ticconi *et al*. 2004). The mutant phenotype of *pdr* suggests that an active

process is essential to protect root meristems against low P conditions; this protective effect may be specific to the lateral root meristems[AS1].

Arabidopsis plans develop a highly branched root system when growing under limiting sulphate (SO_4^{-2}), conditions. Lateral roots are formed closer to the root tip at increased density. NITRILASE3 (NIT3) gene, encodes an enzyme that s able to convert indole-3-acetonitrile to indole-3-acetic acid and is related to limiting SO_4^{-2} conditions, suggesting a direct role for NIT3 in auxin synthesis and root branching (Kutz A et al. 2002).

A number of AUX/IAA genes have been implicated with a sulphur response regulatory element, and that other hormones as ABA and cytokinin are playing a role in sulphur deficiency responses. It have been also suggested that IAA28, auxin influx may modulate the response to low sulphate (Maruyama-Nakashita, A. et al. 2004, Nibau, C. et al. 2008, Nikiforova, V. J. et al. 2005).

The transcriptomic profile of potassium starved plants overlaps with sulphur starvation, but not with nitrate starvation or phosphate starvation, and also involves changes in jasmonate/defence signalling (Armengaud, P. et al. 2004). Interestingly, the MYB77 transcription factor provides a direct link between potassium starvation responses and auxin signalling (Shin, R. et al. 2007).

It will be interesting to understand how these pathways work and make it possible to apply in preparing plants for specific soils and purposes, and so find an optimized balance. Ho, McCannon & Lynch (2003), point out that bean cultivars with shallow root systems are better at obtaining phosphorus, which is located near soil surface, but are therefore probably worse at obtaining water which increases with soil depth.(Ho, Melissa D. et al. 2004)

IV. CONCLUSIONS

As we have seen there is still a lot to study and to understand about the plants physiology. Surely, lateral roots are great scenarios into which discover many complex interactions between the inside/outside plant environment. The plasticity of the root system architecture is playing a huge role in their efficient nutrient and water up-taking system. It will be interesting to understand how these pathways work, and hopefully shortly, use them to make possible elegant genetically-changed plants enhanced for specific soils ready to conquer non-cultivable areas.

The roots are anchored to the substrate and can not choose their environmental conditions. Mineral concentrations in the soil, biotic factors, compactness, porosity and water availability influence the highly regulated pathways cross-talked with phytohormones signals among many others substances. Roots can sense their internal necessities, they aren't isolated from the shoot system, on the contrary the can react very fast to their inside immediate nutrient status, and to external factors as, light, pathogens, gases, etc, acting over the surface.

The field of lateral root development is evolving rapidly. In spite that several processes haven't been cleared, scientists and laics are already trying to improve and adapt profitable plants to available environments and optimize their production.

With a better understanding of those complicated pathways and mechanisms, we will be helping ourselves in many other fields.

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