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Literature Study: Plant growth regulators and signal mechanisms

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Introduction

Plants evolved in the presence of the physical factors on the Earth such as 1-g gravitational force, strong global magnetic field and defined radiation levels for millions of years. They have adapted to these conditions and acquired the ability to use these stimuli to regulate their growth and development. Plant hormones are important molecules transmitting and coordinating a variety of developmental, environmental and metabolic cues. Therefore it is important to understand how the new conditions on the Moon and Mars will affect these powerful molecules and their regulation of plant growth and development.

The five classical classes of phytohormones identified in last half century are auxin, abscisic acid, cytokinin, gibberellin and ethylene. However, during the last 15 years other molecules have also been recognized as hormones, including brassinosteroids, jasmonate, salicylic acid, nitric oxide and strigolactones (Santner and Estelle, 2009).

Auxin represents one of the most important hormone in plants (Bennett *et al.,* 1998). It is involved in development of the embryo, leaf formation, apical dominance, fruit development, abscission, root initiation and development as well as tropisms (Teale *et al.,* 2006). This class of hormone has been the most studied of the all hormones and the knowledge on auxin signalling, biosynthesis and transport has grown immensely in the last years. For these reasons auxin will be the focus of this technical note while the other hormones will be mentioned only when participating in cross-regulatory mechanisms in auxin signalling.

For information on the particular conditions found on the Moon and Mars, see CEAS paper ” Literature Study of Higher Plants in Space for MELiSSA (LiRHiPliSME)- Input to MELiSSA Phase II project” (Kittang *et al.,* 2009).

1. Auxin – Signal mechanisms and regulation of plant growth

1.1 Auxin biosynthesis

The most common form of auxin in plants is IAA (indole-3-acetic acid). The synthesis of IAA can be different depending on plant species, developmental stage and plant organ (Vanneste and Friml, 2009). Young leaves for example have the highest biosynthetic capacity (Ljung *et al.,* 2001).

Auxin biosynthesis is an ambiguous process due to the fact that it can be produced by multiple biosynthetic pathways and also by the fact that a complex metabolic network function to conjugate IAA to sugars, amino acids and peptides or proteins (Delker *et al.*, 2008). IAA is believed to be synthesized in plants through tryptophane (Trp)-dependent pathways and tryptophan-independent pathways. However, our understanding of these pathways is still fragmented since some of the key enzymes and genes involved are unknown. The four Trp-dependent pathways are indole-3-acetamide (IAM) pathway, indole-3-acetaldoxime (IAOx) pathway, tryptamine (TAM) pathway and indole-3-pyruvic acid (IPA) pathway. The biosynthesis of auxin has been reviewed in details by Woodward and Bartel (2005). Here it will be summarized only the principal features of this process.

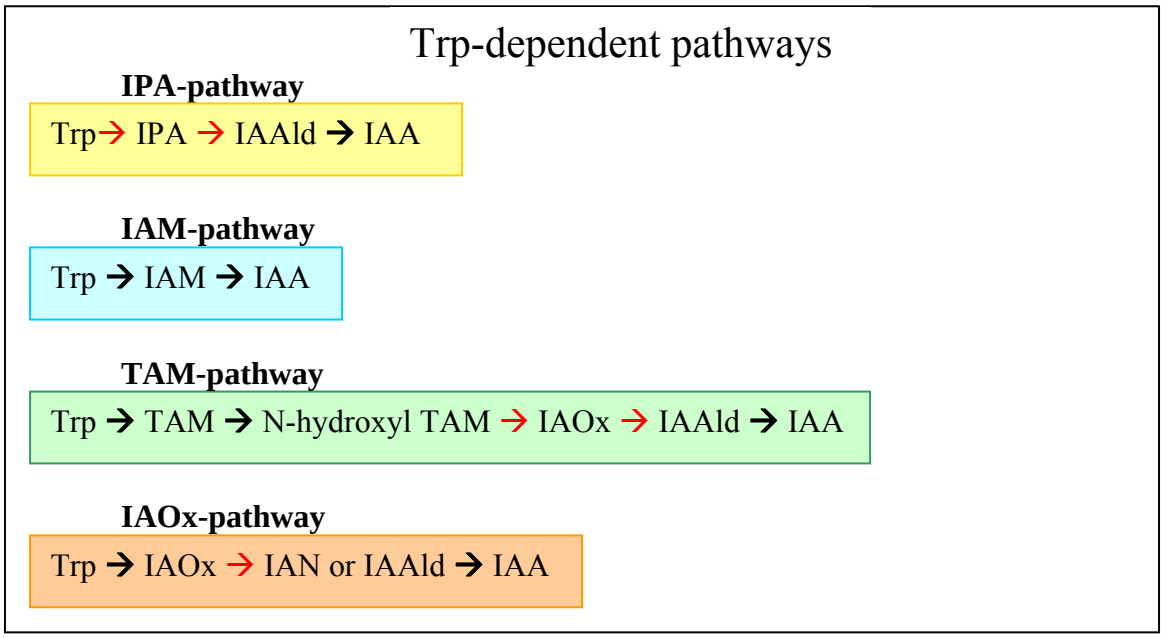

Figure1.1 – The tryptophan-dependent pathways on IAA biosynthesis. The red arrows are accounting for the steps where the enzyme catalyzing the reaction is unknown. The **black** arrows are accounting for the steps where the enzymes are known.

The importance of each of these pathways in plant growth and development is still unclear. So far only the TAM and IPA pathways have been believed to be relevant in plant development (Vanneste and Friml, 2009).

The enzyme involved in the first and the second step of the IPA pathway is unknown (see Figure 1) while aldehyde oxidase protein (AAO1) catalyzes the last step transforming indole-3-acetaldedyde (IAAld) into IAA. The enzymes on the IAM- pathway have been

identified. Trp monooxygenase (IaaM) converts Trp to IAM and IAM hydrolase (IaaH) converts IAM to IAA. The TAM-pathway can also convert Trp to IAA. The enzyme catalyzing the first step is Trp decarboxylase. Later TAM is converted to N-hydroxyl-TAM via Yucca enzymes. The two enzymes catalyzing the next steps have not been identified while AAO1 is known to catalyze the last step. Tryptamine has not been identified in *Arabidopsis*, but the identification of the yucca genes suggests that a tryptamine IAA biosynthetic pathway may operate in plants (Zhao *et al.,* 2001).The IAOx-pathway uses CYP79B2 and CYP79B3 enzymes to catalyze Trp to IAOx. The enzyme oxidizing IAOx to indole-3-acetonitrile (IAN) or IAAld is unknown. AAO1 transforms IAAld to IAA while NIT1, NIT and NIT3 transform indole-3-acetonitrile (IAN) to IAA (Woodward and Bartel, 2005).

Analysis of the Trp mutants has revealed a Trp-independent pathway where the plants can synthesize IAA via Trp precursors as for example indole-3-glycerol phosphate or indole. The importance and existence of a Trp-independent pathway have been questioned by Müller and Weiler (2000). They showed that the increase in IAA conjugates in Trp synthase mutants in fact results from degradation of indole- 3-glycerophosphate, the IAA precursor that hyperaccumulates in the mutants. They believe that mutants may not give accurate information about biosynthetic pathways and the results obtained could be due to an artifact.

Some details about the biosynthesis of IAA remain a mystery that needs a complete enzymatic characterization for clarifying all steps of these processes.

1.2 Auxin signalling

It is well-known that auxin coordinates many plant growth processes such as cell division, elongation and differentiation. But, how auxin signal is perceived and interpreted by plant cells has been a central question in plant biology and it is yet not completely understood.

By genetic studies it was discovered that auxin regulates plant process through transcriptional factors. The auxin transcriptional response is controlled by two large families of transcription factors: the auxin/indole-3-acetic acid (AUX/IAA) and auxin response factor (ARF). ARF proteins bind directly to DNA and can activate or repress the expression of auxin regulated genes. AUX/IAA binds to ARF to repress transcription, acting as negative regulators (Dharmasiri *et al.,* 2005). Finally, auxin regulates transcription by stimulating degradation of AUX/IAA. How auxin actually controlled the degradation of Aux/IAA was a big open question until a few years ago, when Dharmasiri *et al.,* (2005) reported that the F-box protein TIR1 is an auxin receptor that mediates rapid AUX/IAA degradation. It has been shown that auxin binds directly to ubiquitin-

ligase complex SCF ^{TIR1} and promotes the interaction of TIR1 and Aux/IAA. By using crystallographic studies Tan *et al.,* (2007) demonstrated that auxin functions as a molecular glue to enhance TIR1-substrate interaction. Auxin binding to TIR1 does not cause conformation changes. Aux/IAA binds to TIR1 at the same site where auxin is placed (TIR1 'pocket'). Auxin will then increase the affinity between the two proteins (see Figure 1.2).

Figure 1.2 - A model of auxin-regulated TIR1-substrate interaction. Auxin binds to the same TIR1 pocket that docks the Aux/IAA substrate acting as molecular glue. Illustration from Tan *et al.,* 2007

It was clear that other receptors were involved in auxin response after experiments with TIR1 mutants reported only modest effects on plant response to auxin. It was then suggested that the F-box proteins AFB1, AFB2 and AFB3 (Table 1.1) are also auxin receptors involved in the degradation of Aux/IAA (Dharmasiri *et al.,* 2005).

Table 1.1 Auxin receptors

Auxin	
Receptors	**Receptor type**
TIR1	F-box protein
AFB1, AFB2, AFB3	F-box protein

It is yet not clear whether other receptors are involved in auxin signalling or not. But there are indications that other pathways of auxin signalling may exist. For example sometimes the action of auxin occur too fast foe the response made through gene expression. Auxin-binding protein-1(ABP1) has for a long time been believed to be an auxin receptor. ABP1 binds auxin with high specificity and affinity (reviewed in Teale *et al.,* 2006). Loss of ABP1 causes embryonic arrest; inactivation of ABP1 also dramatically impairs plant growth. However, the biological function of ABP1 remains poorly understood and no other components of this signalling pathway have been identified (reviewed in Vanneste and Friml, 2009).

1.3 Mechanism of auxin transport

Auxin is synthesized in young apical tissues, and is then transported to its basal target tissue through two distinct major pathways: One is for rapid, long-distance transport through the phloem. This non-polar transport occurs by loading auxin into the mature phloem and distributing it to the sink tissues, such as the root (Marchant *et al.,* 1999). In the other slower type of transport (ca. 5-10mm per hour), IAA moves from cell to cell and is distributed through a specialized directional manner called polar auxin transport (PAT, Table 1.2).

The directional transport of auxin was discovered by Charles Darwin while studying tropisms in plants. The polar transport became a key feature of auxin. But, how IAA was transported trough this polar manner was a mystery. In 1970, the *chemiosmotic model* was created to explain this specialized transport. IAA is a weak acid, in the cytoplasma IAA is found in an ionic form (IAA^-) that is membrane-impermeable. IAA needs efflux carriers to leave the cytoplasm. On the extracellular matrix (low pH), IAA is protonated (IAAH) and can diffuse freely through the plasma membrane into cells, or by H^+/IAA^- symport mediated by influx carriers (see Figure 1.3) (Robert and Friml, 2009).

The efflux and influx carrier proteins have been identified. AUX1, which belongs to the Auxin permease1/like Aux (AUX1/LAX) family, has been characterized as auxin influx carriers.

Members of the PIN-FORMED proteins have been characterized as auxin efflux carriers. This finding gave strong molecular support to the chemiosmotic theory. The PIN efflux proteins are the most important carriers in the auxin transport machinery because they show polar subcellular localization that correlate with known direction of auxin flow (reviewed in Tanaka *et al.,* 2006). PIN1 was characterized as an efflux carrier, seven other genes similar to PIN1 were found in Arabidopsis. The functionality of the PIN5, PIN6 and PIN8 has not been defined yet. However, PIN2, PIN3, PIN4 and PIN7 have been attributed to function in gravitropism, tropic responses, root meristem patterning and early embryo development (review by Vieten *et al.,* 2007). The PIN proteins have the ability to control the direction of the auxin transport due to their highly dynamic switch in polarity. This switch in polarity is only possible through delivery and recovery of PIN-containing vesicles to and from the plasma membrane enabling a rapid and flexible control of PIN localization (review by Vanneste and Friml, 2009; Fleming, 2006).

Table 1.2 Auxin transporter carriers involved in PAT

Auxin transporter carrier	Type of transport	Protein family
AUX1	Influx	AUX1/LAX
ABCB1/PGP1 ABCB4/PGP4 ABCB19/PGP19	Efflux	ABCB/PGP
PIN [PIN1,PIN2, PIN3, PIN4, PIN7]	Efflux	PIN-FORMED

The dynamical polarity of the PIN proteins has been shown to be a trigger of developmental changes. Different environmental stimuli utilize auxin distribution to execute a specific developmental program. PIN3, for example, has been demonstrated to be responsible for the lateral relocation of auxin efflux in tropisms (Friml *et al.*, 2002). The relocation of auxin due to perception of light or gravity causes asymmetrical growth. In gravitropism the roots will bend. The results found by Friml *et al.* were in agreement with the classical Cholodny-Went model of tropisms (see Figure 1.4). For more details about the role auxin in gravitropism and the effects of gravitropism in plants see Technical Note – Plant movements.

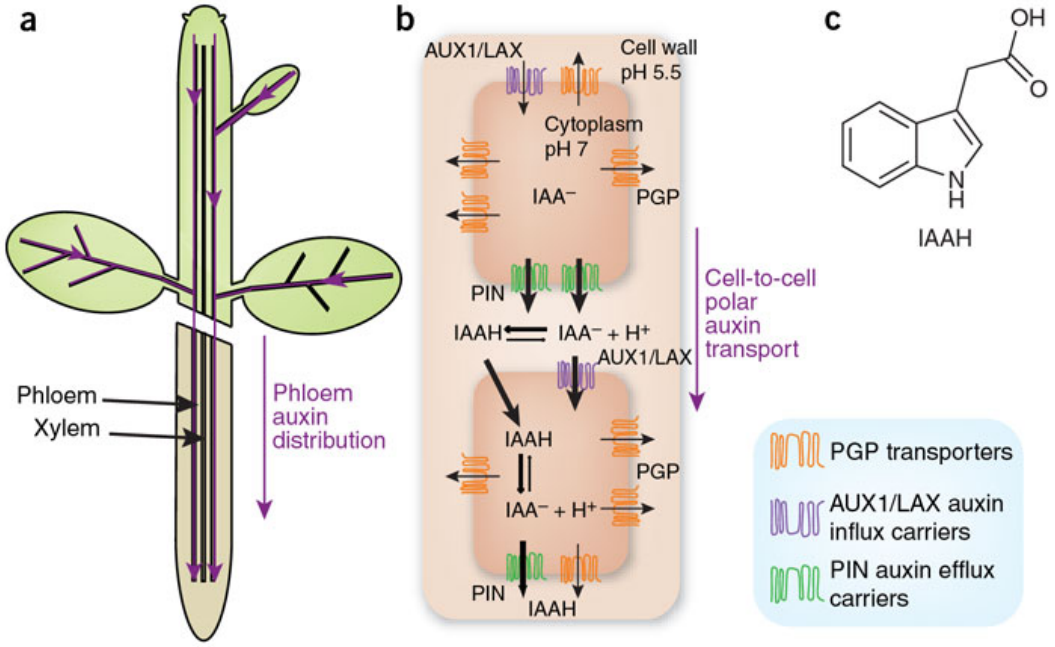

Figure 1.3 - (**a**) Auxin distribution via the phloem to root and shoots. (**b**) The chemiosmotic model, based on the pH difference between the apoplast (pH 5.5) and the cytoplasm (pH 7.0). Protonated auxin—(IAAH)—can diffuse through the lipidic plasma membrane or be transported by the AUX1/LAX influx carriers into the cell. In the neutral cytosol, it is deprotonated to (IAA^-). IAA^- can exit cells by the action of PGP- or PIN-type efflux carriers. The polar cellular localization of the carriers determines the directionality of the intercellular auxin flow. (**c**) Structure of protonated IAAH. Illustration from Robert and Friml (2009).

The diverse PIN transport carriers (Figure 1.4) are responsible for different directions of auxin efflux depending on the tissue and or/stimuli. In root gravitropism PIN1 and PIN4 are responsible for the acropetal transport of auxin. PIN2 accounts for the basipetal transport. PIN7 functions in root acropetal auxin transport. PIN3 is responsible for the lateral distribution of auxin.

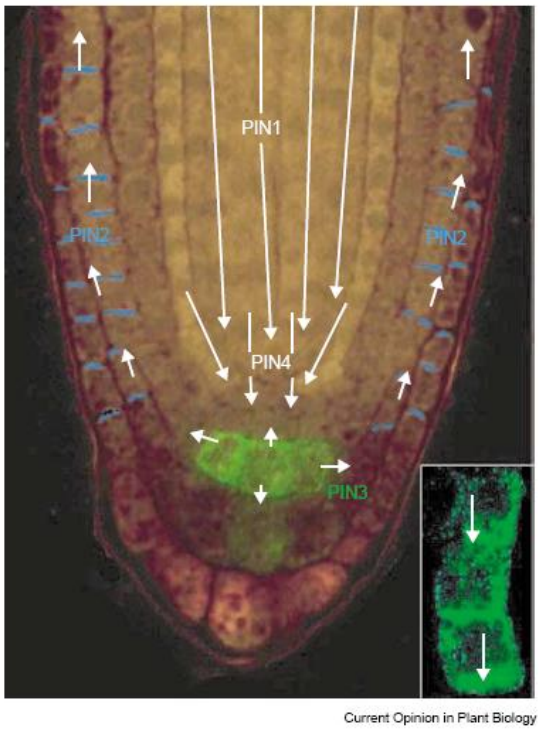

Figure 1.4 – Role of the PIN protein carriers in Arabidopsis root apex and probable routs of PAT. Immunolocalization of PIN3 (green). Auxin is supplied to the root cap by PIN1 and PIN4 (white route). PIN3 distributes auxin laterally depending on the stimuli. Auxin is then further distributed by the PIN2 (blue route). Illustration from Friml (2003).

Other important auxin efflux carriers are the P-glycoproteins of the ABCB/PGC transporter family. The best characterized are ABCB1/PGP1, ABCB4/PGP4 and ABCB19/PGP19. The efflux of auxin through the ABCBs carrier is non-directional. It is believed that the ABCB proteins may control the amount of auxin available in the cells, while PIN proteins are responsible for the vectorial aspect of the intercellular auxin movement (Vanneste and Friml, 2009). Specific interactions between the ABCB and PIN transport carriers appear to be important for the cellular directionality of the transport. Recent data suggested that ABCB19 stabilises PIN1 localisation at the plasma membrane that enhances PIN1 auxin transport activity, especially in mature tissue (Titapiwatanakun *et al.*, 2009).

1.4 Cross-talk between auxin and other plant hormones

Physiological studies suggest various links between plant hormones. Their interaction seems to be crucial in regulating many developmental processes such as cell division, elongation and differentiation. Several hormones regulate or are regulated by auxin. The auxin cross talk has been reviewed in details by Swarup *et al.,* (2002) and only a short summary will be given here.

Cytokinin and auxin regulates plant cell proliferation by controlling the expression of the cell cycles components Cdc2 and CycD3. Auxin and cytokinin control plant development by regulating each other's abundance (Swarup *et al.,* 2002).

Ethylene, in combination with auxin, regulates elongation and apical hook formation of the hypocotyl, root hair differentiation/elongation, and root growth inhibition. For promoting hook elongation ethylene depends on the polar auxin transport. The control of PAT by ethylene is required in order to maintain and form the apical hook. Root growth is inhibited by ethylene, but auxin is also necessary for this regulation process. Application of ethylene to the roots will enhance the auxin synthesis and that will cause a change from longitudinal to radial cell expansion. Auxin will then have a role in the inhibition effects of ethylene. The link between auxin and ethylene here is through enhanced transcription of ACS genes. Auxin also seems to be involved in the ethylene-mediated hypocotyl elongation. Ethylene induces root hair formation, and again auxin play a role in this regulation. But the exact mechanisms by which ethylene, auxin and other hormones influence root hair formation are not clear yet (reviewed by Dugardeyn and Van Der Straeten, 2008).

Auxin and ABA have been observed to interact on the control of the stomata opening. Auxin causes a reduction in turgor in guard cells to open stomata while ABA increases the turgor to close. There is also evidence that ABA and auxin may interact to influence root growth and seed germination. ABA may act modulating the response and/or the transport of auxin in the primordial lateral root developing (Swarup *et al.,* 2002).

Auxin has also been described to co-ordinately regulate several developmental programs in plants with other hormones such as gibberellins (GA), brassinosteroids (BR) and jasmonic acid (JA). PAT is required for the biosynthesis of GA in pea stem tissue. Auxin and GA is believed to coordinate pea stem elongation and positively regulate each other biosynthesis. Auxin and BR positively interact to control lamina joint bending, mediating gravitropic responses. Auxin and JA have been reported to differentially regulate two vacuolar proteins (V*spA* and V*spB*) during early stages of seedling growth (Swarup *et al.,* 2002).

2. Microgravity effects

The direct involvement of auxin in tropism (especially gravitropism) will be covered by the Technical Note 'Plant movement'. The present document focus will be on the direct effects of microgravity on auxin transport and/or synthesis.

2.1 Space flight experiments

There were only two experiments done under space flight conditions regarding the effects of microgravity on plant growth regulators. The first experiment performed by Schulze *et al.,* (1992) reported no difference in the amount of IAA and ABA between *Zea mays* seedlings grown in space and on ground. However, this was a very simple experiment where the seeds were placed in canisters with no environmental control. The results were also originated from post flight analysis.

The second experiment BRIC-AUX was performed on STS-95 by Ueda *et al.,* (2000). They made an analysis of the polar auxin transport in maize coleoptiles and pea epicotyls after 6 days in space flight conditions. In total 64 seeds of pea and maize was distributed into four plant growth chambers but the germination rate and number of plants obtained are not described in the article. The results showed that the polar auxin transport was significantly inhibited in pea and promoted in maize which was found also under simulated microgravity conditions (see section 2.2). The reason for the difference in response between maize and pea has not been clarified yet. But it could be due to differences on the PAT system between plant species. The results also suggest that the PAT or the construction of the system of PAT is considered to be under the control of gravity (Ueda *et al.,* 2000).

The experiment performed by Ueda *et al.,* (2000) was the first important step to understand how auxin and the PAT can be affected by true microgravity. But, there are still many questions regarding the effects of the microgravity environment on plant hormone response that need to be addressed.

2.2 Simulated microgravity

Clinostats have been extensively used to study the effects of gravity on auxin polar transport. But their use as simulation of weightlessness has divided the scientific community. Many scientists believe that the stimuli caused by clinostats can not be considered as microgravity while others consider that clinostats, specially the 3-D, can give results that are similar to that found under microgravity conditions. Therefore, only a short summary of the studies performed using clinostats will be presented here.

The polar auxin transport has been studied using both a horizontal and a 3-D clinostat (see Table 2.1). The polar auxin transport in both *Arabidopsis thaliana* and pea was suppressed under the conditions of simulated microgravity. To the contrary the polar auxin transport of maize was promoted (Oka *et al.,* 1995, Hoshino *et al.,* 2005, Shimazu *et al.,* 2000, Ueda *et al.,* 1999). The results are in agreement with those found during the space flight experiments BRIC-AUX described in Section 2.1.

To understand how microgravity/and simulated microgravity affected auxin transport in pea Hoshino *et al.,* (2005) studied the effects of the 3-D clinostat on the expression of the genes PsAUX1 and PsPIN2 that encodes for auxin influx and efflux carriers in etiolated pea. The experiments were done according to the procedures used by Ueda *et al.,* (2000) during the STS-95 space experiment. The results showed that the expression of PsPIN1 and PsAUX1 genes encoding auxin influx and efflux substantially increased, while the polar auxin transport decreased as already reported by other authors. The reason why the genes encoding for PIN2 and AUX1 were down-regulated is still unclear. However, it could indicate that the expression of these genes is sensitive to gravity. This study also suggests that PAT is dependent on the presence and localization of the transport proteins. The fact here is that gene expression and effectors protein abundance are not linked. Moreover, it is only the case for particular members of the PIN family.

Table 2.1 – Effects of clinorotation on polar auxin transport

Clinostat	Plant species	Effects	Reference
Horizontal (3 rpm)	*Arabidopsis thaliana* (32 days old)	-Reduction of 62% in the length of inflorescence axis -Inhibition of auxin transport	Ishii *et al.*, 1996
Horizontal	*Arabidopsis thaliana* (21 days old)	-Activity of IAA polar transport reduced to 60% of control	Oka *et al.*, 1995
Horizontal (0.5 rpm)	*Arabidopsis thaliana*	-Activity of IAA polar transport reduced to 60% of control	Miyamoto *et al.*, 1999
3-D	Pea and maize (6 days)	-Polar auxin transport was suppressed in pea -Polar auxin transport was increased in maize	Ueda *et al.*, 1999
3-D	Pea (*Pisum sativum*) (6 days)	-Suppressed polar auxin transport -increased gene expression of PsPIN1, PsPIN2 and PsAUX1	Hoshino *et al.*, 2005

3. Radiation Effects

3.1 UV- radiation

Plants grown under enhanced UV conditions have shown changes in growth, general development and flowering. Plant hormones play an important role in many of these plant processes. Alteration in the concentration of the plant hormones may induce changes in the processes regulated by them (Hollósy 2002).

Early reports have already documented a reduction in IAA content in response to UV exposure. Klein (1967) reported reduced auxin levels in *Oryza sativa* while Witztum *et al.*, (1978) reported changes in auxin levels in *Spirodela oligorhiza*. The enhanced UV conditions have also inhibited auxin action in tomato (deZeeuw and Leopold, 1957).

Ros and Tevini (1995) observed reduced stem elongation in sunflower seedlings after exposure to UV-B radiation. The *in vivo* concentration of IAA was reduced by 51% compared to the control. It was concluded that the photolytic degradation of IAA was the cause of the reduced stem elongation in sunflower seedlings. Reduction in IAA content and increase in peroxidase and IAA oxidase activities exposed to enhanced UV-B have

been reported for rice (Huang *et al.,* 1997). Peroxidase is possibly involved in the oxidation of IAA and that may have triggered the decomposition of IAA in rice treated with UV-B (Jansen *et al.,* 2001).

Hormonal changes induced by UV-B in vegetative and reproductive tissues of tomato have been studied by Yang *et al.* (2004). They observed an inhibition of pollen germination, reduction in seed size and delay in seed germination. Also were found differences in hormone content; IAA was reduced, while the concentration of GA was increased. UV-B clearly affected tomato reproduction, and these changes in reproduction may be due to the changes in hormone concentration.

Katerova *et al.* (2009) studied the effects of low doses of UV-B and UV-C radiation on the hormones content in pea seedlings. Seedlings were exposed to 14 consecutive days of low doses of UV-C (0.1 $kJm^{-2}d^{-1}$, 0.3 $kJm^{-2}d^{-1}$) and UV-B (4.4 $kJm^{-2}d^{-1}$, 13.3 $kJm^{-2}d^{-1}$). The UV-C irradiation led to a reduction in ABA content, while UV-B did not alter ABA. Both treatments led to increased aminocyclopropane carboxylic acid (ACC) content, suggesting that the plants were stressed. Surprisingly the UV-C radiation caused an accumulation of IAA (377% compared to control). The UV-B radiation did not affect the IAA content. The results indicate that UV-C radiation can cause more hormonal disturbances than UV-B leading to higher levels of injury. Pea also seems to be more UV-B resistant than rice and tomato, which showed a decrease in the IAA content after exposure to UV-B (Huang *et al.,* 1997; Yang *et al.,* 2004).

3.2 Gamma radiation

Gamma radiation is well-known to provoke retardation and inhibition of plant growth in many plant species (Kovács and Keresztes, 2002; Wi *et al.,* 2005, 2007). On the other hand, low doses of gamma (1 or 2 Gy) can increased plant growth slightly in *Arabidopsis* seedlings (Wi *et al.,* 2007). The link between growth reduction and changes in auxin content has been studied by different researchers. Auxin levels in plants have been shown to be reduced after high doses of irradiation (Cooke, 1955; Gordon, 1957; Moore and Hough, 1962; Kutáček *et al.,* 1966).

Despite the evidence for the involvement of auxin in the growth reduction of gamma irradiated plants, it was still unclear whether the growth retardation was mediated by the change in auxin level or its action. Momiyama *et al.* (1999) investigated the changes in the activity of enzymes related in the biosynthesis of IAA. The results demonstrated that radiation doses lower than 1kGy led to a temporal inhibition of coleoptile elongation. Higher doses of radiation caused a stronger inhibition and cessation of elongation. Lower doses of radiation (< 1kGy) caused a decrease in IAA levels in the coleoptile tips. Higher

levels of radiation also caused damage on the IAA perception of the cells. Momiyama *et al.* (1999) reported that IAA molecules in aqueous solution were easily decomposed by low doses (30 Gy) of radiation. However, in the presence of tissue extract, the decomposition was strongly suppressed, after a delay phase with no effect immediately after irradiation (see Figure 3.1). It was also reported that the activity of IAAld oxidase, an enzyme involved in the biosynthesis of IAA, was reduced only after high dose irradiation of 3kGy.The reduction in the activity of IAAld oxidase is a good track, but does not explain the effects caused by lower irradiation in IAA content. Other mechanisms have also to be considered.

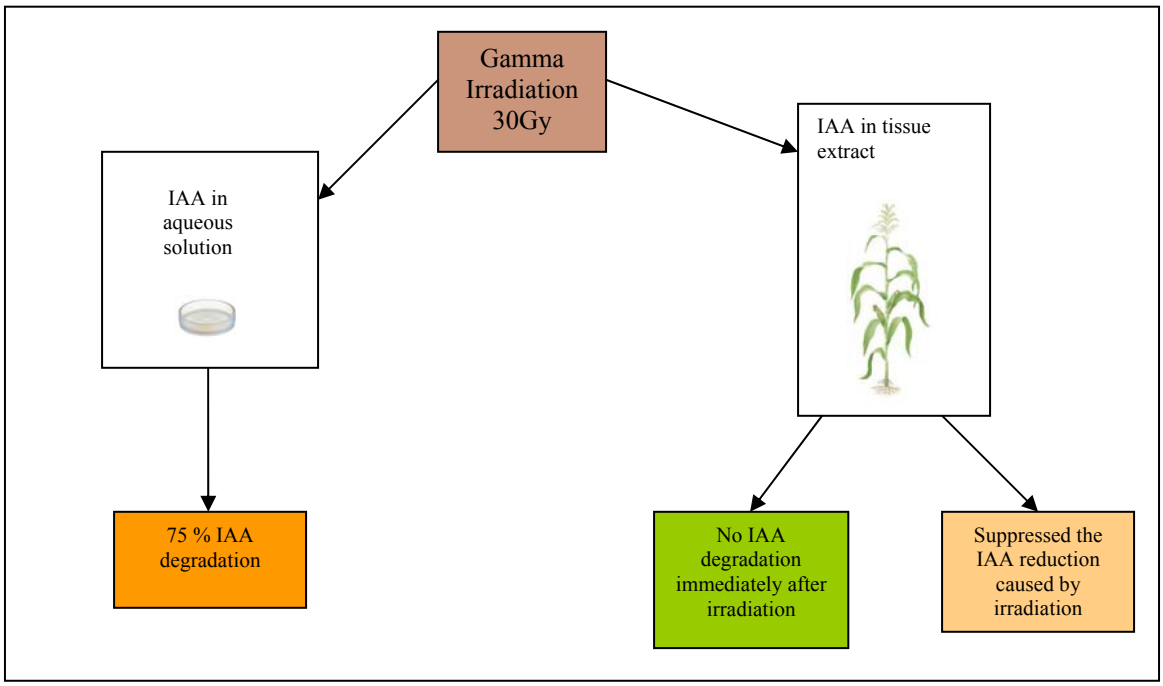

Figure 3.1 – IAA in aqueous solution (physiological concentration) was decomposed after gamma irradiation. The degradation was strongly suppressed in the presence of tissue extract. The tissue extract may contain some natural substances that protect IAA against the radiation. The IAA content on the tissue extract immediately after the irradiation was similar to the control. It could indicate that the delayed effect on IAA resulted not from the direct effect of the irradiation on the IAA molecules, but rather on other processes.

3.3 X-ray

No relevant article was found dealing with the effects of X-ray on plant hormones. Spencer and Cabanillas (1956) reported variations in growth provoked by X-ray in indigo seedlings.

3.4 Heavy ions, protons and neutrons

Most of the literature regarding the effects of radiation on plant hormones deals with ionizing radiation and not neutrons and protons. Due to their neutral charge, neutrons are not affected by magnetic fields. Neutrons can penetrate deeper in materials causing extensive damage; neutrons can also be produced by secondary particles. On the International Space Station (ISS) the neutron radiation has been estimated to be 20 percent of the total radiation (Koshiishi *et al.,* 2002). In addition to that, no significant protection against neutrons has been found yet. Therefore it is of vital importance to know the effects of these particles on higher plants.

Spencer and Cabanillas (1956) reported variations in growth provoked by neutrons irradiation ($7.91x10^{12}n/cm^2$ and $9.84x10^{13}n/cm^2$) in indigo seedlings (*Indigofera endecaphylla*). The reduction in growth was believed to be due to reduced auxin activity.

More recently, Fortunati *et al.* (2008) reported that neutron radiation affects the expression of genes involved in the response to auxin in *Arabidopsis thaliana***.** The results showed notable variations in up- or down-regulation of ARFs and Aux/IAA expressed genes that are involved in auxin transport. In the wild-type the ARF genes were down-regulated as well as the Aux/IAA3 and Aux/IAA6. The expression of AUX1 and EIR1 was clearly down-regulated. For the mutants (transport of auxin) the results were reversed. The effects were stronger with lower irradiation dose (48.8 mGy) than with higher (76 mGy). The plants showed a partial recovery after 48 hours.

Studies with low energy heavy ions have been performed by Yang *et al.,* 2008. Due to their low energy the nuclei of the heavy ions will stop into the sample causing well localized damages, especially near the surface. Arabidopsis seeds were irradiated with 1.5×10^7 ions/cm^2 (30 keV), and the bystander effects caused by radiation were analyzed. The results showed that long-term and short-term postembryonic development was inhibited. It was concluded that the inhibition was caused by bystander effects since shoot apical meristem and root apical meristem were not directly affected by the radiation. Treatment with synthetic auxin or reactive oxygen species (ROS) reversed the effects. It was suggested that radiation had a direct impact on auxin homeostasis though auxin synthesis. It was concluded that ROS- and auxin-dependent transcriptional processes may be affected by radiation causing the bystander effects.

No articles were found about the effects of proton radiation on plant hormones.

3.5 Space radiation

No articles were found regarding the effects of space radiation (performed on ISS or Space Shuttle) on plant hormones. The BRIC experiments with pea and maize (Ueda *et al.*, 2000) were done for 6 days under space flight conditions, but the effects of space radiation could not been discriminated by the effect of microgravity since no on-board reference centrifuge was used.

4. Magnetic field effects

4.1 Weak magnetic field effects

In different experiments effects of a weak magnetic field (WMF; with shielding of the geomagnetic field) on plant growth have been reported. Weak magnetic field (10nT-3 weeks) was capable of delaying plant development in barley (Lebedev *et al.,* 1977). In other experiments a strong inhibition in root growth was reported in sugar beet, wheat and pea exposed to WMF. However after 4 days in WMF the plants seemed to recover from the inhibition (Sytnik *et al.,* 1984).

Some scientists suggest the involvement of auxin in the growth inhibition caused by weak magnetic field. However, no evidence has been found yet.

4.2 Magnetic field in addition to GMF

Magnetic fields have been reported to exert a positive effect on plant growth and development. Plants from magnetically treated tomato seeds (100-170 mT) were greater than control plants (De Sousa *et al.,* 2006). Chronic exposures of rice seeds to magnetic fields (150-250mT) have been reported to increase the rate and percentage of germination (Carbonell *et al.,* 2000). Moon and Chung (2000) demonstrated that magnetic treatment improves germination rate and first stages of growth of bean and wheat plants. Martinez *et al.* (2000) showed that magnetic fields (125mT) increased the length and weight of barley seeds. Magnetic field is believed to enhance the activation of seed-store auxin leading to an improvement in vegetative growth and yield (De Sousa *et al.,* 1999). Auxin is also believed to be involved in positive effects of magnetic fields on whole plants.

Polar auxin transport (PAT) is dependent on ion fluxes (see Section 1.3) and ions, due to their charge, can be easily affected by magnetic fields. It can be realistic to assume that magnetic fields affect PAT. However, more experiments need to be done to clarify the mechanisms involved in this pathway.

5. Conclusion

Our knowledge on the effects of microgravity, radiation and magnetic fields on plant hormones is limited.

Experiments in simulated and in the true microgravity environment, under space flight conditions, have showed that auxin content is affected by microgravity (see Section 2.0). However, only one experiment has been performed in space, which makes it difficult to draw a relevant conclusion.

Enhanced UV-B, UV-C, and gamma irradiation have been shown to decrease auxin levels in plants, leading to growth inhibition (see Section 3.1 and 3.2). But, many open points still have to be addressed. More experiments need to be done is this area.

There are some indications that neutrons can induce variation in growth and can also affect the expression of genes involved in auxin transport. Low energy heavy ions are believed to induce bystander effects in irradiated seeds; auxin seems to play a role in the response to these effects (see Section 3.4). However, only few experiments have been performed.

Despite the great number of indications of the role of auxin in the growth variations induced by magnetic fields and weak magnetic fields, there is still no confirmation of a conclusive hypothesis. More experiments need to performed, in order to understand the link between auxin, growth variation and magnetic fields.

6. Recommendations for further work

There are several questions that need to be answered regarding the effects of microgravity, radiation and magnetic fields on plant hormones/auxin.

- Experiments on how interactions of auxin metabolism with other hormones are affected by microgravity need to be conducted. The network is directing plant growth; auxins do not stand as such. That is the main research question. Which part of the PAT fails in microgravity? Is that failure at the gene expression level? Is the inhibition of PAT in pea only transitory? Will the plants adapt to microgravity or reduced gravity levels?

- Experiments regarding the effects of space radiation on auxin are required. There are no experiments done in space.

- More ground experiments regarding the effects of different types of radiation, such as neutrons, protons, heavy ions, gamma on hormones signalling and content, are needed. It is important to classify the effects of radiation to be able to differentiate the damages caused by diverse types of radiation found under space conditions.

- The interactions between auxin/PAT and magnetic fields need be understood better, since they might influence plant growth in a weak magnetic field (Moon and Mars).

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