Physiology of Auxin in Response to Environmental Stress and Heavy Metal Pollution

Dissertation

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By

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Abstract

Phyto-hormones play critical roles in the coordination of plant growth and development. Generally, plant hormones promote, inhibit, or qualitatively modify plant growth and development. This complex process requires signal transduction, specific information pathways, translating intra- or extra-cellular signals into specific cellular responses within a cell. Auxins represent an important class of plant hormones. Plant growth is regulated by auxin via the regulation of various genes responsible for different metabolic processes in the plant. The impact of toxic heavy metals like cadmium and transition metals like copper and manganese in the soil, on plant defence reactions and their implications for plant growth have been extensively investigated. However, how auxin is involved in mediating environmental stresses and heavy metal stress is still not understood.

This study was designed to address the following questions in poplar: Are there changes in the expression pattern of GH3::GUS promoter-reporter construct in relation to seasonal changes under natural field conditions? What is the effect of cadmium stress on the auxin physiology? What is the effect of excessive manganese on auxin physiology? What are the copper requirements for poplar growth? Poplar was chosen for this study as it is a model forest tree for molecular studies. Grey poplar, *Populus* x *canescens* (a hydrid of *P. tremula* and *P. alba*) and three transgenic lines transformed with a GH3::GUS construct responsive to auxin, were used. The plants were grown in compost soil outdoors as well as in hydroponic media under greenhouse conditions to investigate auxin pattern in the tissues.

Populus x *canescens* grown in soil was studied during one year from autumn 2006 until summer 2007 and auxin physiology responded seasonally as indicated by significant changes in the GUS expression along the stem axis both within and during the different seasons. To detect changes of the GUS along the stem axis, the GUS expression was evaluated by comparing the GUS activity in each portion of the stem with the GUS activity in the other portions in every season. To investigate changes of GUS activity during different seasons, GUS activity for each part of the stem was compared with the GUS activity in the same part in the other seasons. Significant changes in GUS activity in all the stem regions (except the stem apex) were found during the different seasons. Measurements of gas exchange, biomass and dry mass and growth parameters (leaf formation, stem diameter, plant shoot height and side shoot formation) showed that the wildtype, WT and the transgenic lines, TG were similar. This indicated that transformation did not disturb the general physiology of the plants. Positive correlations were observed between the day length and both growth and auxin patterns. It was concluded that seasonal changes induced changes in the auxin pattern.

Cadmium treatment (50 µM Cd) resulted in growth inhibition, reduction of auxin in the stem apex and an increase of the GUS expression downwards along the stem. These data indicate that cadmium interfered with auxin physiology and suggested that cadmium severely diminished auxin biosynthesis in the stem apex. One possibility for the decreased auxin level in the apex after Cd treatment might be due to a direct effect of Cd on auxin, e.g. by inducing its break down. Cadmium analysis in different plant tissues showed an increase in cadmium content after addition of cadmium in the growth medium. *Populus* x *canescens* showed substantial amounts of Cd accumulated in the roots in response to cadmium treatment after 24 days of exposure. The concentration of Cd retained by the roots was 11 times higher and 44 times higher than the concentration of Cd in the stem and leaves, respectively. Thus, roots restricted Cd transport to the above ground part. This could be a tolerance mechanism for *P.* x *canescens* when grown in metal-contaminated sites. Generally, cadmium caused alteration in auxin biosynthesis and auxin distribution patterns, which suggests a direct link to growth regulation.

Excessive Mn treatment (10 mM Mn) had no clear effect on the growth of *Populus* x *canescens.* Seemingly, the plants were Mn-tolerant during the treatment of 14 days. GUS staining did not show a consistent effect on the pattern along the stem. However, Mn decreased the GUS activity in the root tissues. This suggests that Mn altered the auxin physiology in the root more than in the shoot.

The effect of copper treatments (0 – 500 µM Cu) on growth, physiological and biochemical parameters was investigated. Suppression of growth, damage to photosystem II and inhibition of the activities of stress enzymes, guaiacol peroxidase and NADH oxidase were observed at concentrations from 50 to 500 µM Cu. Generally, *Populus* x *canescens* exhibited high sensitivity to copper stress.

This study demonstrates that growth and auxin physiology of *Populus* x *canescens* were influenced by both environmental factors and heavy metal stress. The GH3::GUS construct was successfully employed for characterising and mapping the auxin physiology in poplar stems. Cadmium and manganese have different effects on auxin physiology of *Populus* x *canescens.* More work needs to be done to find out whether heavy metals like cadmium and manganese act directly on auxin or on its biosynthesis.

Zusammenfassung

Phytohormone spielen eine wichtige Rolle in der Steuerung des Wachstums und der Entwicklung von Pflanzen, in dem sie verschiedene Prozesse unterstützen, verhindern oder modifizieren. Diese komplexen Prozesse erfordern Signaltransduktion, spezifische Informationswege sowie die Umwandlung von intra- oder extrazellulären Signalen in spezifische Zellantworten. Eine wichtige Klasse von Phytohormonen sind die Auxine. Auxine regulieren das pflanzliche Wachstum über die Regulation verschiedener Gene, die verschiedene Stoffwechselwege steuern. Auf der anderen Seite beeinflussen äußere Stressfaktoren wie Schwermetalle, z.B. Cadmium oder Übergangsmetalle, z.B. Kupfer und Mangan ebenfalls das pflanzliche Wachstum. Hierzu gibt es zahlreiche Untersuchungen. Über den Zusammenhang oder die Einbindung von Auxinen in die Stressantwort von Pflanzen auf verschiedene Stressbedingungen, wie z. B. Schwermetallstress ist dagegen bisher weniger bekannt.

In dieser Arbeit wurden folgende Forschungsfragen an der Versuchspflanze Pappel untersucht:

1. Ändert sich das Expressionsmuster von GH3:GUS Promotor-Reporter-Konstrukten im jahreszeitlichen Wechsel unter natürlichen Bedingungen?
2. Welchen Einfluss hat Cadmium auf die Auxin-Physiologie?
3. Welchen Einfluss haben hohe Konzentrationen von Mangan auf die Auxin-Physiologie?
4. Wie beeinflusst Kupfer das Pappelwachstum?

Pappel wurde verwendet, da sie die Modellpflanze für molekularbiologische Studien im Forstbereich ist. Für die Analyse der Auxinmuster in den verschiedenen Pflanzengeweben wurde die Graupappel, *Populus* x *canescens* (ein Hybrid aus *P. tremula* und *P. alba*) and drei transgene Linien mit einem GH3::GUS Konstrukt verwendet. Die Pflanzen wuchsen entweder in Komposterde unter Außenbedingungen oder in hydroponischem Medium unter Gewächshausbedingungen.

Die Auxin-Physiologie in *Populus* x *canescens*, gewachsen in Komposterde, wurde im jahreszeitlichen Gang zwischen Herbst 2006 und Sommer 2007 untersucht. Die Auxin-Physiologie wurde indirekt über die GUS-Expression innerhalb des Stammes und in Abhängigkeit von der Jahreszeit gemessen.

Die GUS-Aktivität unterschied sich in allen Bereichen des Stammes (mit Ausnahme des Apex) zwischen den verschiedenen Jahreszeiten signifikant. Die Tageslänge korrelierte positiv mit dem Pflanzenwachstum und den Auxin-Mustern. Jahreszeitliche Wechsel führen

vermutlich auch zum Wechsel der Auxin-Muster. Wildtyp-Pflanzen und transgene Pflanzen zeigten ähnliche Werte im Gaswechsel, in der Biomasse, im Trockengewicht und in verschiedenen anderen Wachstumsparametern (Blattbildung, Stammdurchmesser, Pflanzenhöhe und Seitensprossbildung). Dies zeigt, dass die Transformation der Pappeln nicht zu morphologischen Veränderungen der Pflanzen führte.

Cadmium-Behandlung (50 µM Cd) führt zur Wachstumsreduktion der Pflanzen und zur Reduktion der GUS-Expression (als Maß für die Auxinmenge) in apikalen Stammbereichen bzw. zur Erhöhung in unteren Stammbereichen. Diese Ergebnisse weisen auf einen Zusammenhang zwischen der Auxin Physiologie und Cadmium hin. Cadmium führt entweder zur Reduktion der Auxin-Biosynthese in apikalen Stammbereichen oder zu einem verstärkten Auxin-Abbau. Die Zugabe von Cadmium in das Wachstumsmedium führte zu erhöhten Cadmium-Mengen in den verschiedenen Pflanzengeweben. *Populus* x *canescens* zeigt stark insbesondere stark erhöhte Cadmium-Konzentrationen in den Wurzel nach einer 24 stündigen Behandlung. Die Cadmium-Konzentration war etwa 11-fach höher als im Stamm und etwa 44-fach höher als in den Blättern. Dies bedeutet, dass der Transport von Cadmium von den Wurzeln zu den oberen Pflanzenteilen eingeschränkt ist, welches einen möglichen Toleranzmechanismus darstellen könnte.

Hohe Mangan-Konzentrationen im Medium (10 mM Mn) haben keine eindeutigen Effekte auf das Wachstum von Populus x canescens. Vermutlich sind die Pflanzen tolerant gegenüber der 14-tägigen Behandlung. Die GUS-Aktivität im Stamm zeigte ebenfalls keine eindeutigen Veränderungen durch die Mangan-Behandlung. Dagegen war die GUS-Aktivität in den Wurzeln verringert. Mangan beeinflusst daher die Auxin-Physiologie in den Wurzeln stärker als im Spross.

Der Effekt verschiedener Kupferkonzentrationen (0-500 µM) auf das Wachstum und verschiedene physiologische und biochemische Parameter bei Pappel wurde untersucht. Kupferkonzentrationen zwischen 50 und 500 µM reduzieren das Wachstum, schädigen Photosystem II und hemmen die Aktivität von stressinduzierten Enzymen wie der Guajakol-Peroxidase oder der NADH abhängigen Oxidase. *Populus* x *canescens* reagiert allgemein sehr empfindlich auf erhöhte Kupferkonzentrationen.

Die Ergebnisse dieser Arbeit zeigen, dass das Wachstum von und die Auxin Physiologie in *Populus* x *canescens* stark von Umweltfaktoren und Schwermetallen beeinflusst wird. GH3::GUS Konstrukte konnten erfolgreich verwendet werden, um die Auxin Physiologie in Stämmen von Pappel zu charakterisieren. Cadmium und Mangan beeinflussen die Auxin Physiologie in *Populus* x *canescens* in unterschiedlicher Weise.

Weitere Untersuchungen sind notwendig, um die Frage zu beantworten, ob Schwermetalle direkt auf Auxin wirken oder auf die Biosynthese von Auxin.

Abbreviations

2,4-D = 2,4-Dichlorophenoxyacetic acid
µ = micro.
ABA = abscicic acid.
ANOVA = analysis of variance.
AuxRE = auxin response element.
b = bar.
BCA = bicinchoninic acid reagent.
BSA = bovine serum albumine.
BSP = bark storage protein.
Ca = calcium.
Cd = cadmium.
CF = chlorophyll fluorescence.
CF = methyl-2-chloro-9-hydroxyflurene-9-carboxylate.
cm = centimetre.
Cu = copper.
d = day
DCP = 2,4- dichlorophenol.
ddH_2O = double distilled water.
DMF = dimethylformamid
DW = dry weight.
EC = electrolyte conductivity.
e-cup = eppendorf tube.
EDTA = ethylenediamine tetraacetic acid.
et al = et alia (Latin) = and others.
FAE = formaldehyde: acetic acid: ethanol
F´= basic fluorescence in light
F´m= maximum fluorescence in light.
F0= basic fluorescence in darkness.
Fm= maximum fluorescence in darkness.
FM= fresh mass

FW = fresh weight.

g = gram.

Glutathione-POD = glutathione peroxidase.

Guaiacol-POD = guaiacol peroxidase.

GR = glutathione reductase

GSH = glutathione peroxidase (reduced form)

GSSH = glutathione peroxidase (oxidized form)

h = hour.

HRP = horseradish peroxidase.

IAA = indole-3-acetic acid.

IBA = indole-3-butyric acid.

ICP–AES = induced coupled plasma atomic emission spectroscopy.

Kg = kilogram.

KPP = kalium phoshpate puffer.

L = litre.

LAM = lateral auxin movement.

m = metre.

max. = maximum.

mg = milligram.

ml = millilitre.

mRNA = messenger RNA.

M = molar (mol/l).

min. = minute.

mm = millimetre.

Mn = manganese.

MPa = mega pascal.

NAA = naphthaleneacetic acid.

NADH-oxidase = NADH oxidase.

nm = nanometre (wave length units).

No. = number.

NPA = naphthylphthalamic acid

PAR = photosynthetically active radiation.

PAT = polar auxin transport.

PCR-cycle = photosynthetic carbon reduction cycle.

PCs = phytochelatins.
PSI = photosystem I.
PSII = photosystem II.
P- value = probability value.
PVP = polyvinylpolyrrolidone.
REC = relative electrolyte conductivity.
RH = relative humidity.
ROS = reactive oxygen species.
r.p.m = rotation per minute.
RT = room temperature.
s = second.
SD = standard deviation.
S-D plants = short-day plants.
SH = sulphur hydryl group.
SOD = superoxide dismutase.
TG = transgene.
TIBA = 2, 3, 5-triiodobenzoic acid.
UV = ultra violet.
v/v = volume/volume.
w/v = weight/volume.
WT = wildtype
X-Gluc = 5-bromo-4-chloro-3-indolyl-ß–D-glucoronic acid.
Q_A = primary electron acceptor.
$\Phi PSII_{dark}$= maximum quantum yield of photosystem II.
$\Phi PSII_{light}$= actual quantum yield of photosystem II in light conditions.

List of companies

Company	**City**	**Country**
Arthur Pfeiffer Hochvakuum Technik GmbH	Wetzlar	Germany
Beckman Instrument Inc.	Fullerton	USA
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Chapter 1

General Introduction

1.1 Environmental pollution by heavy metals

Pollution of the environment constitutes a great threat to the well-being of humankind and other biological communities. Heavy metals are important environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons (Benavides *et al*. 2005). Because they cannot be degraded or destroyed, heavy metals are persistent in all parts of the environment. Heavy metals are increasing in the soils as a consequence of industrial activity world-wide. Mining wastes, fertilisers, paper mills and toxic elements from atmospheric emissions, have all contributed to the continuous deposition resulting in substantial accumulation of toxic metals in the environment (Gratao *et al*. 2005). For instance, cadmium is a strong stress agent, and plants are exposed to such pollutants increasingly often under conditions of growing anthropogenic pressure on the environment (Seregin and Ivanov, 2001). The heavy metal pollution of the soil is exacerbated by the fact that heavy metals accumulated in plants may, either directly or indirectly, find their way into animals and human beings (Pal *et al*. 2006).

Heavy metals enlist a relatively large series of elements with the specific density over 5 g/ cm^3 and the relative atomic mass above 40. Many of these metals manifest high affinity for sulphur-containing ligands and strongly bind the latter. Therefore, when such heavy metals among them e.g. Cd enter the cell, they interact with SH-groups, inactivating many enzymes and disturb many metabolic processes (Seregin and Ivanov, 2001). Some heavy metals such as Fe, Mn, Zn, Ni and Cu are required in plants as essential micro-nutrients whereas others such as Hg, As and Cd act as toxic compounds (Polle and Schützendübel, 2003). Heavy metals have detrimental effects on plant growth and survival. Based on their chemical and physical properties three different molecular mechanisms of heavy metal toxicity can be distinguished: (a) production of reactive oxygen species (ROS) by autoxidation and Fenton reaction, a typical reaction for transition metals like copper and manganese, (b) blocking of essential functional groups in biomolecules, a reaction mainly reported for non-redox-reactive heavy metals such as cadmium, and (c) displacement of essential metal ions from biomolecules, a reaction that occurs with different kinds of heavy metals (Schützendübel and Polle, 2002).

Gratao *et al*. (2005) reported that exposure to toxic metals can intensify the production of reactive oxygen species (ROS), compared with unstressed plants. Some of the ROS species are highly toxic and must be detoxified by cellular protective systems, if the plant is to survive and grow. It is also well established that excess concentrations of the heavy metals significantly affected plant water status, causing water deficit and subsequent changes in the plants (Kastori *et al*. 1992). Plants – like all other organisms possess homeostatic cellular mechanisms to maintain the correct concentrations of essential and other metal ions inside the different cellular compartments and to minimize the potential damage that could result from the exposure to non-essential metal ions (Benavides *et al*. 2005; Clemens, 2001). Mycorrhizas, which ensheath root tips help to avoid metal toxicity (Marschner,1995; Jentschke and Godbold, 2000).

Solutions are urgently needed to address the serious environmental contamination by toxic metals. The most commonly used methods for dealing with heavy metal pollution are still extremely costly. Phytoremediation, the use of plants to extract, sequester and/or detoxify pollutants is a new and powerful technique for environmental clean-up (Memon *et al*. 2001). In the process of phytoremediation the plants are introduced into an environment and accumulate the contaminants in their roots and leaves. Phytoremediation is sometimes termed `*green technology*` and it is considered an environmentally friendly technology, that is a safe and also a cheap way to remove contaminants (Gratao *et al*. 2005).

Some studies (Sawidis *et al*. 1995; Aksoy *et al*. 2000) used trees as biomonitors to evaluate air pollution by heavy metals. Samecka-Cymerman *et al*. (2004) compared the efficiency between *Phragmites communis*, *Salix viminalis* and *Populus canadensis* in removing the pollutants (heavy metals and the macro-elements) from sewage in subsurface flow constructed wetlands in Poland. The authors described the effectiveness of the observed phytoremediation system investigated as insufficient alone to remove these elements and can be considered as a supplemental tool in purification of sewage.

Some plants that tolerate metal-contaminated soils such as some cultivars of *Brassica jucea* (L.) Czern. possess a strong ability to accumulate large amounts of heavy metals in their roots and shoot. This property may be exploited for soil reclamation. It was suggested that these plants may be used to clean up toxic metal-contaminated sites (Kumar *et al*. 1995). Clonal variation in poplar was investigated by Laureyesens *et al*. (2004) for heavy metal uptake and accumulation and biomass production in 13 different poplar clones in a short rotation coppice culture established on a former waste disposal site, moderately polluted with heavy metals. They found significant clonal differences in accumulation for most metals taken up. Moreover, the lowest concentration was found in wood, the highest concentrations were generally found in scenescing leaves, making removal and treatment of fallen leaves necessary. In a similar study, fast-growing trees *Populus tremula* and *Salix viminalis* L. showed appreciable potential for storing heavy metals in ageing foliage (Hermle *et al*. 2007). Thus, these species are considered well suited to phytoremediate heavy metal-contaminated soils.

1.2 Cadmium

The toxic effects of cadmium on plants have been extensively investigated. Excess Cd^{2+} causes a number of toxic symptoms in plants, e.g. growth retardation, inhibition of photosynthesis, induction and inhibition of enzymes, altered stomatal action, disturbed water relations, efflux of cations, and generation of free radicals (Prasad, 1995). In 10-d-old bean plants (*Phaseolus vulgaris* L. cv Contender) treated with 3 μM Cd^{2+} for 6 days, it was observed that Cd^{2+} induced an inhibition of the expansion growth of bean leaves. The authors refuted the assumption that a decrease in turgor to be the cause of this inhibition; they postulated that decreased cell wall extensibility may be a cause of the reduced cell expansion (Poschenrieder *et al*. 1989). In hydroponically-grown *Arabidopsis thaliana,* the Cd^{2+} effects ($CdCl_2$, 10 – 100 μM for 1 week) were investigated. It was proposed that Cd^{2+} affects guard cell regulation in an ABA-independent manner by entering the cytosol via Ca^{2+} channels (Barbeoch *et al*. 2002).

1.3 Copper and manganese

Copper (Cu) is a transition metal and an important micro-nutrient for normal plant growth and development. Manganese (Mn) is also an essential micronutrient for the plant. Unlike cadmium, Cu and Mn are redox active elements. Copper and manganese, although beneficial at sub-toxic levels, they can induce extensive apoptosis in yeast (*Saccharomycetes cerevisiae*) cells via different pathways when present in excess (Liang and Zhou, 2007). Many studies showed that these micronutrients may cause oxidative stress when present in excess in the environment (Ducic and Polle, 2005; Schützendübel *et al*. 2002; Babu *et al*. 2003; Gonzalez *et al*. 1998). Oxidative stress is a central factor in abiotic and biotic stress phenomenon that occurs when there is a serious imbalance in any cell compartment between the production of reactive oxygen species (ROS) and the antioxidant defence, leading to physiological challenges (Foyer and Nector, 2000). Reactive oxygen species like hydrogen peroxide and superoxide anion radicals are produced in both unstressed and stressed cells. They are involved in both oxidative stress and the plant defences against such stress. Plants have well-developed defence systems against ROS, involving both limiting the formation of ROS as well as instituting its removal. In this respect, peroxidases, PODs play an important role (Shi *et al*. 2006; Arora *et al*. 2002). Under unstressed conditions, the formation and removal of O_2 are in balance. However, the defence system, when presented with increased ROS formation under stress condition, can be overwhelmed (Alscher *et al*. 2002).

Free radicals and other active derivatives of oxygen are inevitable by-products of biological reactions. Reactive oxygen species inactivate enzymes and damage important cellular components. The increased production of toxic oxygen derivatives is considered to be a universal or common feature of stress conditions (Arora *et al*. 2002). The activity of the antioxidative defence system must be equal to the task of destruction of ROS in normal metabolism and at times when the plant suffers stress. A study of Yu and Rengel (1999) in narrow-leafed lupin demonstrated that Cu, Zn and Mn deficiency decreased the concentration of these elements in lupin leaf tissue and depressed plant growth. Cu, Zn or Mn altered the activities of SOD forms depending on the kind and severity of deficiency stress, indicating that oxidative stress is one of the components of micronutrient deficiency stress. The authors postulated that manipulation of the capacity of plants to tolerate oxidative stress may influence their capacity to tolerate micronutrient deficiency.

1.4 Auxin

1.4.1 Functions of auxin

Phytohormones play critical roles in the coordination of plant growth and development (Takahashi *et al*. 1995). Auxins were the first plant hormones discovered and they represent an important class of plant hormones (Takahashi *et al*. 1995; Srivastava, 2001; Swarup, 2001). Auxin is derived from the Greek word *auxien* which means to grow or increase. Generally, plant hormones promote, inhibit, or qualitatively modify plant growth and development. This complex process requires signal transduction, specific information pathways, translating intra- or extra-cellular signals into specific cellular responses within a cell (Tiryaki, 2004). The first auxin isolated was indole-3-acetic acid, IAA, the most abundant naturally occurring auxin. Indole-3-acetic acid, IAA is the endogenous entity of auxin, a plant hormone that controls various physiological processes in plants including apical dominance, tropism, shoot elongation and root initiation (Matsuda *et al*. 2005).

1.4.2 Regulation of auxin

Since IAA plays a dominant and essential role in controlling virtually all aspects of plant growth and development, the level of IAA in the plant tissues should be properly regulated. The mechanisms by which plants regulate levels of the phytohormone IAA are complex (Clere *et al*. 2002; Rampey *et al*. 2004). Endogenous IAA concentrations are regulated by three main processes: biosynthesis, degradation, and conjugation (Normanly *et al*. 1995). Genetic and biochemical experiments have demonstrated both tryptophandependent and tryptophan-independent routes of IAA biosynthesis (Bartel, 1997; Bartel *et al*. 2001). Plants produce active IAA both by de novo synthesis and by releasing IAA from conjugates such as IAA-Aspartate and IAA-Glutamate (Bartel, 2005). In plants, the growth regulator IAA is found both free and conjugated to a variety of amino acids, peptides and carbohydrates (Bartel and Fink, 1995; Seidel *et al*. 2006). Most of IAA in plants is maintained in conjugated forms (Cohen and Bandurski, 1982). The IAA conjugates play multiple functions in IAA regulation in the plant. IAA conjugates help in the transport of IAA, may serve as temporary storage reservoirs of inactive IAA that can be hydrolyzed to provide the plant with active hormone, protection of IAA from enzymatic destruction, and in the homeostatic control of the concentration of IAA in the plant (Bartel, 1997; Clere *et al*. 2002; Cohen and Bandurski, 1982). Bialek and Cohen (1992) investigated the role of amide-linked IAA conjugates in germinating seedlings of beans, *Phaseolus vulgaris*. The authors suggested a more general role for IAA conjugates in the control of seedling growth than simply to serve as a seed storage form of auxin. Some studies have suggested a relationship between the biological activity of IAA-amino acid conjugates and their hydrolysis rates. This correlation was inferred from the study of IAA conjugates as slow release forms of auxin in plant tissues. The released IAA in turn, determines the availability of IAA to the growing, dividing and differentiating cells (Hangarter and Good, 1981; Bialek *et al*. 1983).

1.4.3 Auxin transport

Auxin is transported in the plant body in a polar fashion. The polar movement of auxin from the young leaves towards the roots through pro-cambium, cambium or parenchyma tissues triggers a complex sequence of changes that ultimately results in the formation of a vascular strand along the flow of auxin. Once developed, this vascular strand remains the preferable pathway of auxin transport, in as much as cells possessing the ability to transport auxin are associated with the vascular tissues (Aloni, 1987). Recently, Schrader *et al*. (2003) isolated the putative polar auxin transport genes, *PttLAX1-PttLAX3* and *PttPIN1-PttPIN3*, belonging to the AUX1-like family of influx and PIN1-like efflux carriers, respectively. Analysis of *PttLAX* and *PttPIN* expression suggested that specific positions in a concentration gradient of the hormone are associated with different stages of vascular cambium development and expression of specific members of the auxin transport gene families. At the end of the growing season, the auxin transport capacity was severely diminished, paralleled by reduced expression of *PttLAX* and *PttPIN* genes. The authors concluded that trees have developed mechanisms to modulate auxin transport in the vascular meristem in response to developmental and environmental cues. In an adaptive response that allows trees to survive