J Plant Growth Regul (2004) 23:1–8
DOI: 10.1007/s00344-004-0076-5

J P G R
Journal of Plant Growth Regulation

From Auxin Homeostasis to Understanding Plant Pathogen and Plant Symbiont Interaction: Editor's Research Interests

Jutta Ludwig-Müller*

Institut für Botanik, Technische Universität Dresden, 01062, Dresden, Germany

Introduction

To introduce myself as the new Editor-in-Chief of the *Journal of Plant Growth Regulation* I wish to briefly describe my research interests. In the following, I present a short overview of current research projects in my laboratory. Although this article is written in review style and provides a short theoretical background, my aim was not to present a comprehensive review of the subject. Therefore, the literature I have cited should not be considered a complete list.

Plant hormones – of Course!

Plant hormones, as we all know, are important substances for growth and development of plants, but they are also important factors for the interaction of plants with microorganisms during pathogenesis or symbiosis. In my laboratory, we are mainly interested in the role of auxins in plant developmental processes as well as signaling molecules during pathogenesis and symbiosis.

Received: 12 December 2003; accepted: 17 December 2003; Online publication: 19 May 2004

*Corresponding author; e-mail: Jutta.Ludwig-Mueller@mailbox.tu-dresden.de

Auxin Homeostasis

Auxins are a class of plant hormones that, at low levels, control a plethora of developmental processes but inhibit plant growth at higher concentrations. Therefore the regulation of hormone concentration is crucial for the proper development of plants. The concentration of a given hormone can be regulated by 1) biosynthesis, 2) reversible inactivation, 3) degradation and 4) transport.

Over 95% of the total auxin in a plant can be found in the conjugated form, therefore the formation of auxin conjugates is one of the key regulatory pathways for the activation/inactivation of IAA (Hangarter and Good 1981; Cohen and Bandurski 1982; Bandurski and others 1995). Conjugates can be formed with amino acids or sugars, but the formation of conjugates with peptides and proteins has also been described (Bialek and Cohen 1986; Bandurski and others 1995; Walz and others 2002).

Amide conjugates account for the bulk of conjugated indole-3-acetic acid (IAA) in dicots studied to date. Various IAA conjugates, among them IAA-Aspartate, IAA-Glutamate, IAA-Alanine, and IAA-Leucine, have been identified as natural conjugates in several plant species including *Arabidopsis thaliana* (Cohen 1982; Östin and others 1992; Barratt and others 1999; Tam and others 2000; Kowalczyk and Sandberg 2001).

IAA amidohydrolases are thought to control the quantity of IAA that is released from the conjugated

into the free form (Cohen and Bandurski 1982; Bandurski and others 1995). Several IAA amidohydrolases have been isolated from *Arabidopsis thaliana* (Bartel and Fink 1995; Davies and others 1999). Because auxin conjugate hydrolases have not been well characterized in other plant species, a homolog of the *A. thaliana* ILR1 hydrolase was isolated from the close relative *Arabidopsis suecica* and dubbed sILR1 (Campanella and others 2003). Examination of the enzymatic characteristics of sILR1, when overexpressed in *E. coli*, showed a different substrate specificity compared to ILR1, indicating probable differing functions. In contrast to ILR1, the sILR1 protein can utilize IAA-Alanine and IAA-Glycine as substrates more effectively than ILR1 but cannot cleave IAA-Phenylalanine or IAA-Leucine as substrates (Campanella and others 2003; LeClere and others 2002). Auxin conjugate hydrolases identified from a partially purified protein extract from Chinese cabbage seedlings also showed preference for IAA-Alanine but an additional activity, which was able to cleave IAA-Aspartate, was discovered in root extracts infected with the clubroot pathogen *Plasmodiophora brassicae* (see below; Ludwig-Müller and others 1996). To learn more about the structure - substrate relationship we have begun to clone and characterize more hydrolase genes/proteins from different plant species including monocots such as wheat and the model legume *Medicago truncatula*.

In addition to the small molecular weight conjugates, IAA conjugated to peptides and proteins may play a role in auxin homeostasis. Recently, Walz and others (2002) suggested that IAA-amino acid conjugates are present at low levels whereas IAA bound to peptides and proteins account for the majority of IAA-amide conjugates. A gene for a bean (*Phaseolus vulgaris*) 42 kDa protein with IAA covalently attached has been isolated. The protein shows homology to a seed protein from *Glycine max*. However, the physiological role of such a protein is yet unclear. Two possibilities have been proposed: 1) storage form of IAA in seeds and 2) a novel protein modification by the plant hormone. Functional analysis of the gene will shed light on this question. To this end, we have begun to transform model plants (*Arabidopsis, Medicago truncatula*) with this gene from bean and also to isolate IAA-modified proteins from *Arabidopsis*.

Although IAA is considered the major native auxin in higher plants and therefore also the most studied auxin (Normanly and others 1995), other substances with auxin activity, including 4-chloroindole-3-acetic acid, IBA, and phenylacetic acid (PAA), occur in plants (Ludwig-Müller 1999a). Recently we have shown the occurrence of IAA, IBA and PAA in nasturtium (*Tropaeolum majus* L.) (Ludwig-Müller and Cohen 2002). Although IBA is a very prominent auxin in horticultural practice, it was only recently recognized as a native compound in various species such as maize (*Zea mays* L.) and *Arabidopsis thaliana* (reviewed in Ludwig-Müller 2000a). It was demonstrated that IAA is converted to IBA *in vivo* using dark-grown maize seedlings (Ludwig-Müller and Epstein 1991). The same reaction occurs in *Arabidopsis thaliana* seedlings and mature plants (Ludwig-Müller and Epstein 1994) and two varieties of sterile cultured *Grevillea* (Ludwig-Müller 2003), but not in apple cuttings and stem slices (van der Krieken an others 1992, 1993). IBA may be formed by the acetylation of IAA with acetyl-CoA in the carboxyl group, because in labeling experiments the carboxy-^{14}C was retained (Ludwig-Müller and Epstein 1991).

The *in vitro* conversion of IAA to IBA in maize seedlings is catalyzed by a microsomal membrane preparation, which forms IBA in the presence of acetyl CoA and ATP (Ludwig-Müller and others 1995a). The formation of IBA from IAA is not only found in maize, but in a wide variety of other plants (Ludwig-Müller and Hilgenberg 1995). The enzyme involved in the conversion of IAA to IBA in maize, termed IBA synthetase, has been characterized and partially purified. The regulation of IBA synthesis in maize has been studied under different conditions. Induction of IBA synthetase activity was found 1) after exposing the plants to drought and osmotic stress (NaCl and sorbitol) and 2) treatment with abscisic acid (ABA) (Ludwig-Müller and others 1995b), 3) after application of herbicides of the cyclohexanedione type (Ludwig-Müller and others 2000a) and 4) after inoculation with the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* (see below; Ludwig-Müller and others 1997a).

Several reports show that IBA is also metabolized to conjugates. However, contrary to the conjugation patterns of IAA in dicots, the occurrence of substantial amounts of IBA glucose in addition to IBA amide conjugates was described in several dicotyledonous plant species after feeding with labeled or unlabeled IBA (Ludwig-Müller and Epstein 1993; Ludwig-Müller 2000a). We have now begun to characterize the enzymes/genes involved in IBA hydrolysis.

Secondary Plant Metabolites and Auxin Synthesis

The biosynthesis of IAA in Brassicaceae (for example, *Arabidopsis thaliana*) is closely connected to the

synthesis of a group of secondary plant metabolites, the glucosinolates. Glucosinolates are secondary plant products synthesized by members of the Brassicaceae but also occurring in several other plant families (Rodman 1991). They can be grouped into three different classes, depending on the amino acid from which they are derived: 1) aliphatic/alkenyl glucosinolates derived from methionine, 2) aromatic glucosinolates derived from phenylalanine and tyrosine, and 3) indole glucosinolates derived from tryptophan (Bennett and Wallsgrove 1994; Bennett and others 1995). The glucosinolates have been implicated in plant defense against bacterial and fungal pathogens as well as insect predators (Bennett and Wallsgrove 1994), but they may also play a role in host/pathogen recognition.

Indole-3-methylglucosinolate is thought to be an intermediate as well as a storage form for IAA in Brassicaceae. However, other pathways to IAA bypassing the glucosinolates have been described (Hull and others 2000). During a screen for *Arabidopsis thaliana* mutants defective in glucosinolate synthesis, six mutants with different glucosinolate patterns compared to the wild type were isolated (Haughn and others 1991). Four of these six had altered levels of alkenyl glucosinolates, whereas one mutant showed, in addition, low leaf indole glucosinolate concentrations (TU8). This mutant was used to study IAA synthesis during seedling and plant development as well as after pathogen infection (Ludwig-Müller and others 1999). The line TU8 shows traits of an auxin-deficient shoot phenotype (shorter stems, altered branching pattern) as well as rosette size. However, IAA synthesis and metabolism were essentially unaffected in the TU8 mutant during early development (Ludwig-Müller and others 1999).

The development of the clubroot disease of the Brassicaceae (see below) is closely linked to the synthesis of plant hormones such as auxins and cytokinins. Increased cell division and elongation result in the formation of typical root galls (Ludwig-Müller 1999b). The breakdown of indole glucosinolates, which could lead to the release of relatively large amounts of auxin, may be one factor responsible for clubroot symptoms (Butcher and others 1974). This implies that plants with reduced levels of indole glucosinolates should have reduced disease symptoms. It was shown that the mutation TU8 decreased the size of root galls after infection with *Plasmodiophora brassicae* and that the IAA and indole-3-acetonitrile (a precursor of IAA) and indole glucosinolate levels were lower in clubs of the mutant compared to the wild type (Ludwig-Müller and others 1999). Thus, indole glucosinolates and auxin may be pathogenicity factors involved in gall formation.

In a different investigation we were able to show that levels of nitrilase, the enzyme catalyzing the conversion of indole-3-acetonitrile to IAA, were increased in infected roots of *Arabidopsis* and that the nitrilase protein was closely associated with pathogenic structures (Grsic-Rausch and others 2000). In addition, a nitrilase mutant and plants transformed with one of the nitrilase genes in the antisense direction showed delayed club development (Grsic-Rausch and others 2000; Neuhaus and others 2000).

Links Between Auxin and Stress Physiology?

In addition to the phenotypes described above, the TU8 mutant was found to be remarkably less thermotolerant than wild-type plants upon exposure to elevated temperatures. Although a moderate temperature increase only affected shoot growth, exposure to severe heat stress led to a dramatic decay of mutant plants. Survival above optimal temperature conditions is mainly dependent and accompanied by a massive accumulation of heat stress proteins (Hsps), most of which belong to a group of proteins termed molecular chaperones (Ellis and van der Vries 1991). The main task of these proteins is to assist in the refolding of partially unfolded or denatured proteins occurring under elevated temperatures (Forreiter and Nover 1998). Deficiency in expression of chaperones often results in increased thermosensitivity or death of the organism even under normal growth conditions (Lindquist 1986; Nover 1991).

This led us to investigate the expression of heat stress proteins (Hsps) in the mutant under heat stress and control conditions. Although the expression for small Hsps, Hsp70 and Hsp104 was not affected in the TU8 mutant, a remarkable reduction in Hsp90 compared to wild type was found after exposure to elevated temperatures (Ludwig-Müller and others 2000b). Immunolocalization showed that cytosolic Hsp90 is affected. Transient expression of Hsp90 in mutant protoplasts increased their survival rate at higher temperatures to near the equivalent of wild type protoplasts. These data suggest that the reduced level of Hsp90 in TU8 mutants may be the primary cause for the observed reduction in thermostability.

The function of Hsp90 in plants is not yet entirely clear. It has been shown that Hsp90 in yeast and animal cells is involved in the maturation of steroid receptors and several kinases. Our goal is to identify the TU8 gene by map-based cloning and to reveal its

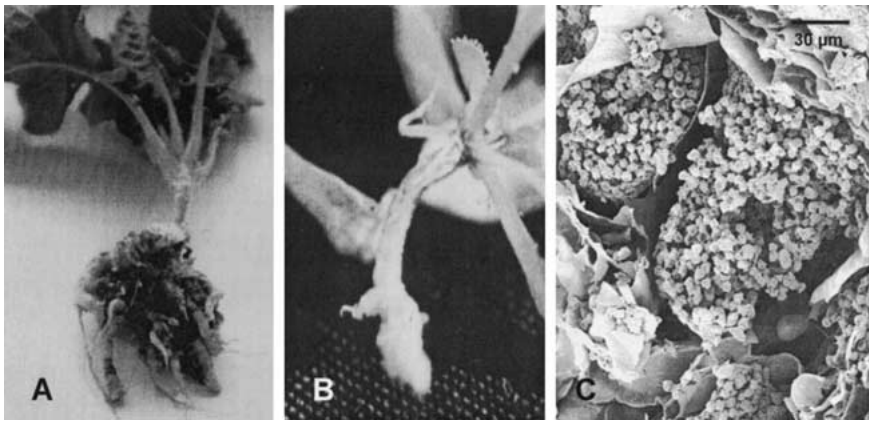

Figure 1. The infection of Brassicaceae with the obligate biotrophic protist *Plasmodiophora brassicae*. (**A**) Infection of a young *Brassica campestris* plant. (**B**) Infection of *Arabidopsis thaliana*. (**C**) Resting spores of the pathogen in a hypertrophied host root cell. Scanning electron microscopy was done in collaboration with Manfred Ruppel, Goethe-Universität Frankfurt, Germany.

function (Wenke and Ludwig-Müller 2002). We are also interested in the extent to which Hsp90 is involved in the apparent mutant phenotype of TU8 plants and whether Hsp90 plays a role in the plant signal transduction network together with plant hormones.

Other indications for an involvement of temperature stress in auxin homeostasis comes from the work of Oetiker and Aeschbacher (1997), who showed that temperature-sensitive cells of henbane (*Hyoscyamus muticus* L.) grow like the wild type at 26°C but die rapidly at 33°C unless auxin is added to the medium. In addition, the conjugates formed from IAA differed in the wild type and temperature-sensitive variant. Although the wild type converted IAA mainly to IAAsp, the variant produced mainly IAA-glucose.

Plasmodiophora Brassicae

The clubroot disease of the Brassicaceae (Figure 1) is probably the most damaging plant disease within this family. About 10% of Brassica field plots worldwide are infected with clubroot and 100% losses were reported from fields infested with *Plasmodiophora brassicae*. As an obligate biotroph, *P. brassicae* completes its life cycle within the host roots (Figure 1C). Only the resting spores of the pathogen can be detected outside the plant tissue. It is difficult to control clubroot in the field because the spores, which are liberated from decomposing root tissue, can remain infectious for at least 15 years and the disease is difficult to control by either chemical or cultural means. Therefore, it is of great importance to understand the development of this disease.

Not only are widely used agricultural cultivars infected with the protist, but weed crucifers such as *Arabidopsis thaliana* are also suitable hosts (Figure 1A,B). Infection with *P. brassicae* induces cells to first divide and during the later stages show signs of hypertrophy (Ingram and Tommerup 1972). The developing clubs form a strong metabolic sink within the host.

Clubroot symptoms clearly suggest that plant growth regulators are involved in disease development. Hyperplasia and hypertrophy, as well as the growth of leaf-like structures from the roots, indicate the involvement of auxins and cytokinins. In a number of studies it was shown that the concentrations of auxins and cytokinins are increased in infected tissue. Although it was demonstrated that the vegetative secondary plasmodia of the pathogen produce cytokinins (Müller and Hilgenberg 1986), the increase of IAA might be due to increased synthesis and turnover of putative host auxin precursors in infected roots (Searle and others 1982; Rausch and others 1983; see above). In addition, other plant hormones such as jasmonic acid may be involved in signal transduction and increased IAA biosynthesis (Grsic and others 1999). Another possible explanation for increased auxin concentrations is the hydrolysis of auxin conjugates in clubroots of Brassica (see above; Ludwig-Müller and others 1996). We were able to clone auxin conjugate hydrolases homologous to *Arabidopsis* hydrolases (Bartel and Fink 1995, Davies and others 1999) from *P. brassicae*-infected *Brassica rapa* roots (Schuller and Ludwig-Müller 2002). Their expression during disease development is currently being analyzed to learn more about the role of auxin conjugate hydrolysis during this host/pathogen interaction. In addition, these auxin conjugate hydrolases will be further characterized with respect to their substrate specificity.

Different features for characterizing the host/pathogen interaction in clubroot have been assessed in the past, namely the degree of colonization of root hairs (Sammuel and Garret 1945; Channon and others 1964), damage induced by the parasite

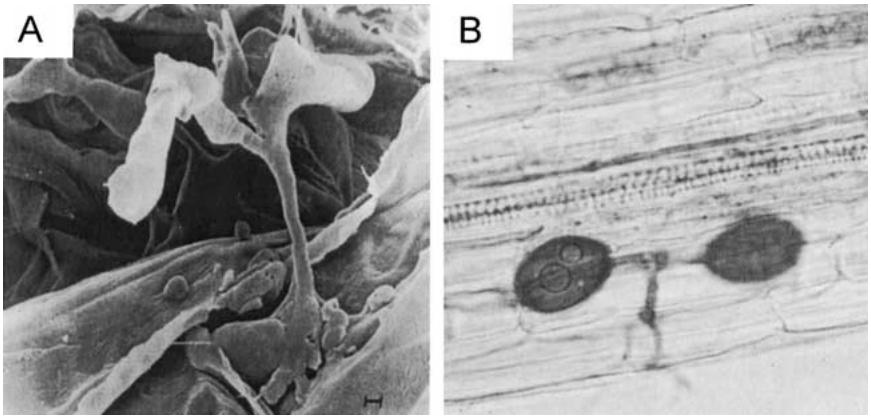

Figure 2. Colonization of maize (*Zea mays* L.) roots with the arbuscular mycorrhizal fungus *Glomus intraradices*. (**A**) Scanning electron micrograph of a young arbuscule (picture was taken in collaboration with Manfred Ruppel, Goethe-Universität Frankfurt, Germany). (**B**) Light microscopy picture of vesicles stained with toluidine blue (picture was taken in collaboration with Dr. Michael Kaldorf, Universität Leipzig, Germany).

measured by weight of shoots and roots or yield loss, and the extent of gall formation (Crute 1986). Most commonly, a subjective scale has been used to grade the size of galls on a population of plants (Buczacki and others 1975). However, different investigations used different methods. Therefore, we assessed the effect of various environmental factors, inoculum density and mutations in *Arabidopsis* and described symptom development by several criteria. The scoring systems we used included disease classes (Fuchs and Saristan 1996; Kobelt and others 2000) and a root index (the relation of root weight of inoculated plants to non-inoculated plants) (Ludwig-Müller and others 1999). Both disease index and root index gave comparable results and can be used for investigations of tolerance or resistance (Siemens and others 2002). Using these scoring systems under defined experimental conditions, a set of *Arabidopsis* mutants with defects in primary and hormone metabolism as well as developmental mutants, which could be involved in clubroot development, was investigated. However, this screen did not reveal many new candidates for tolerance against clubroot.

With *Arabidopsis thaliana* as a host plant for *Plasmodiophora brassicae* we have used the 22 K microarray (Affymetrix) to investigate host gene expression during the development of the disease (Ludwig-Müller and others 2003). Two time points, which were significantly different from each other, were chosen. At the early time point (10 days after inoculation) small secondary plasmodia of the pathogen are visible, but only about 20% of the host tissue is colonized, with limited change to host cell and root morphology. At a later time point (23 days after inoculation) different developmental stages of the pathogen are present. More than 60% of the host root cells were colonized and the root morphology was drastically altered. At both time points more than 1000 genes were differentially expressed when mRNA from infected roots was compared with that from control roots of the same age. However, only a few genes showed similar expression patterns at both time points. Cytokinin oxidase was down-regulated at both time points, but much more strongly at the second time point. In addition, genes, which may function in IAA metabolism (such as members of the GH3 family) or signal transduction, were found to be up-regulated. The defense response of the host plant is modulated by *P. brassicae* as evidenced by the high number of down-regulated defense genes. We hypothesize that *P. brassicae* is able to circumvent the defense response of the host plant by a yet unknown mechanism.

Arbuscular Mycorrhizal Symbiosis

Arbuscular mycorrhiza (AM) is a unique symbiotic association between plant roots and a group of fungi from the order Glomales. This association often increases growth and yield of many crops by enhancing nutrient uptake, resistance to drought and salinity, and increasing tolerance to pathogens (Gianinazzi-Pearson 1996; Mosse 1957; Nelsen and Safir 1982; Smith and Gianinazzi-Pearson 1988). During colonization, distinct structures are formed by the AM fungi within the host roots (internal hyphae, arbuscules, vesicles; Walker 1992) (Figure 2). The complex cellular relationship between host roots and AM fungi requires a continuous exchange of signals, which leads to the proper development of mycorrhiza in the roots of a host plant (Gianinazzi-Pearson 1996). Plant hormones may be suitable candidates for the regulation of such a symbiosis. Little is known about the function of plant hormones during the colonization process al-

though there is evidence that they are involved in signaling events between AM fungi and host plants (Barker and Tagu 2000; Ludwig-Müller 2000b). In addition, it has been suggested that phytohormones, such as IAA and cytokinins, released by mycorrhizal fungi may also contribute to the enhancement of plant growth (Frankenberger Jr. and Arshad 1995).

In maize roots colonized with a *Glomus* isolate, abscisic acid (ABA) levels were considerably higher in AM-colonized than in control roots, whereas the concentrations of zeatin riboside (with the exception of later stages of AM development) and indole-3-acetic acid (IAA) were similar for infected and non-infected roots (Danneberg and others 1992). Hyphae of *Glomus intraradices* were reported to contain ABA, thus contributing to the hormone concentration in colonized roots (Esch and others 1994).

Since stable isotope-labeled standards became available for the determination of plant hormones, it is possible to accurately measure auxin levels using gas chromatography-mass spectrometry (Cohen and others 1986; Sutter and Cohen 1992). With these sensitive techniques, the endogenous content of IAA and indole-3-butyric acid (IBA) in mycorrhizal and control roots of maize was re-investigated (Ludwig-Müller and others 1997a). For IAA, the results of Danneberg and others (1992) were confirmed. However, a comparison of IBA concentrations in mycorrhizal maize roots and controls showed significant differences (Ludwig-Müller and others 1997a). In younger roots, free IBA differed little between infected and control roots whereas amounts of conjugated IBA were higher in the infected tissue. In older roots, the free IBA content was lower in AM-infected roots than in controls, whereas the bound IBA fraction remained the same in infected and control tissues.

An altered phenotype of maize roots inoculated with *Glomus* was found in very young seedlings (10 days after inoculation). We were able to show that the phenotype induced by AM-fungal colonization could be mimicked by exogenous application of IBA and that a synthetic IBA analogue was able to suppress both the IBA-induced and AM-induced root phenotype of young maize plants. In accordance with these findings, the number of fungal structures also decreased in these treated roots (Kaldorf and Ludwig-Müller 2000). IBA is probably involved in lateral root formation to increase the sites for possible fungal infection because it was shown that the preferred sites for penetration were lateral roots compared to main roots (Karabaghli-Degron and others 1998). Changes in root morphology after colonization with arbuscular mycorrhizal fungi have been described for other plant species (see for example, Schellenbaum and others 1991; Yano and others 1996; Tisserant and others 1996). Clearly, our understanding of the role of plant hormones during mycorrhizal symbiosis is still limited, but the application of techniques such as reporter genes, microarrays and transgenic plants will allow us to further dissect the signaling pathways.

ACKNOWLEDGEMENTS

I would like to thank the numerous members of my group who have contributed to this work over the years and also all my collaborators. Work in my laboratory was funded by the Deutsche Forschungsgemeinschaft (DFG) and the Sächsische Landesamt für Umwelt und Geologie (SLUG).

REFERENCES

Bandurski RS, Cohen JD, Slovin JP, Reinecke DM. 1995. Hormone biosynthesis and metabolism: Auxin biosynthesis and metabolism. In: Davies PJ, ed. Plant hormone: physiology, biochemistry and molecular biology 2nd edn., Boston: Kluwer Academic Publishers.

Barker SJ, Tagu D. 2000. The roles of auxins and cytokinins in mycorrhizal symbiosis. J Plant Growth Regul 19:144–154.

Barratt NM, Dong W, Gage DA, Magnus V, Town CD. 1999. Metabolism of exogenous auxin by *Arabidopsis thaliana*: Identification of the conjugate N_a –(indol-3-ylacetyl)-glutamine and initiation of a mutant screen. Physiol Plant 105:207–217.

Bartel B, Fink G. 1995. ILR1, an amidohydrolase that releases active indole-3-acetic acid from conjugates. Science 268:1745–1748.

Bennett R, Wallsgrove R. 1994. Secondary metabolites in plant defence mechanisms. New Phytol 127:617–633.

Bennett R, Ludwig-Müller J, Kiddle G, Hilgenberg W, Wallsgrove R. 1995. Developmental regulation of aldoxime formation in seedlings and mature plants of Chinese cabbage (*Brassica campestris* ssp. *pekinensis*) and oilseed rape (*Brassica napus*): Glucosinolate and IAA biosynthetic enzymes. Planta 196:239–244.

Bialek K, Cohen JD. 1986. Isolation and partial characterization of the major amide-linked conjugate of indole-3-acetic acid from *Phaseolus vulgaris* L. Plant Physiol 80:99–104.

Buczacki ST, Toxopeus H, Mattusch P, Johnston TD, Dixon GR, Hobolth LA. 1975. Study of physiologic specialization in *Plasmodiophora brassicae*: Proposals for attempted rationalization through an international approach. Trans Br Mycol Soc 65:295–303.

Butcher DN, El-Tigani S, Ingram DS. 1974. The role of indole glucosinolates in the clubroot disease of the Cruciferae. Physiol Plant Pathol 4:127–141.

Campanella JJ, Ludwig-Müller J, Bakllamaja V, Sharma V, Cartier A. 2003. ILR and sILR1 IAA amidohydrolase homologs differ in expression pattern and substrate specificity. Plant Growth Regul 41:215–223.

Channon AG, Flint AE, Hinton RAL. 1964. A quantitative laboratory for inoculating cabbage seedlings with *Plasmodiophora brassicae* Woron. Ann Appl Biol 54:71–76.

Cohen JD. 1982. Identification and quantitative analysis of indole-3-acetyl-L-aspartate from seeds of *Glycine Max* L. Plant Physiol 70:749–753.

Cohen JD, Bandurski RS. 1982. The chemistry and physiology of the bound auxins. Annu Rev Plant Physiol 33:403–430.

Cohen JD, Baldi BG, Slovin JP. 1986. $^{13}C_6$-(benzene ring)-indole-3-acetic acid: A new internal standard for quantitative mass spectral analysis of indole-3-acetic acids in plants. Plant Physiol 75:257–260.

Crute IR. 1986. The relationship between *Plasmodiophora brassicae* and its hosts: The application of concepts relating to variation in inter-organismal associations. Adv Plant Path 5:1–52.

Dannenberg G, Latus C, Zimmer W, Hundeshagen B, Schneider-Poetsch H-J, Bothe H.. 1992. Influence of vesicular-arbuscular mycorrhiza on phytohormone balances in maize (*Zea mays* L.). J Plant Physiol 141:33–39.

Davies R, Goetz D, Lasswell J, Anderson M, Bartel B. 1999. IAR3 encodes an auxin conjugate hydrolase from *Arabidopsis*. Plant Cell 11:365–476.

Ellis RJ, Vries SM van der . 1991. Molecular chaperones. Annu Rev Biochem 60:321–347.

Esch H, Hundeshagen B, Schneider-Poetsch H-J, Bothe H. 1994. Demonstration of abscisic acid in spores and hyphae of the arbuscular-mycorrhizal fungus *Glomus* and in the N2-fixing cyanobacterium *Anabaena variabilis*. Plant Sci 99:9–16.

Forreiter, C, Nover, L (1998) The heat stress response and the concept of molecular chaperones. J. Biosci 23:287–302.

Gianinazzi-Pearson V. 1996. Plant cell responses to arbuscular mycorrhizal fungi: getting to the roots of the symbiosis. Plant Cell 8:1871–1883.

Fuchs H, Sacristan MD. 1996. Identification of a gene in *Arabidopsis thaliana* controlling resistance to clubroot (*Plasmodiophora brassicae*) and characterization of the resistance response. Mol Plant Microbe Interact 9:91–97.

Grsic S, Kirchheim B, Pieper K, Fritsch M, Hilgenberg W, Ludwig-Müller J. 1999. Induction of auxin biosynthetic enzymes by jasmonic acid and in clubroot diseased Chinese cabbage plants. Physiol Plant 105:521–531.

Grsic-Rausch S, Kobelt P, Siemens J, Bischoff M, Ludwig-Müller J. 2000. Expression and localization of nitrilase during symptom development of the clubroot disease in *Arabidopsis thaliana*. Plant Physiol 122:369–378.

Hangarter RP, Good NE. 1981. Evidence that IAA conjugates are slow release sources of free IAA in plant tissues. Plant Physiol 68:1424–1427.

Haughn GW, Davin L, Giblin M, Underhill EW. 1991. Biochemical genetics of plant secondary 25 metabolites in *Arabidopsis thaliana*. The glucosinolates. Plant Physiol 97:217–226.

Hull AK, Vij R, Celenza JL. 2000. Arabidopsis cytochrome P450s that catalyze the first step of tryptophan-dependent indole-3-acetic acid biosynthesis. Proc Natl Acad Sci USA 97:2379–2384.

Ingram DS, Tommerup IC.. 1972. The life history of *Plasmodiophora brassicae* Woron. Proc Royal Soc Lond B 180:103–112.

Kaldorf M, Ludwig-Müller J. 2000. AM fungi might affect the root morphology of maize by increasing indole-3-butyric acid biosynthesis. Physiol Plant 109:58–67.

Karabaghli-Degron C, Sotta B, Bonnet M, Gay G, le Tacon F. 1998. The auxin transport inhibitor 2,3,5-triiodobenzoic acid (TIBA) inhibits the stimulation of *in vitro* lateral root formation and the colonization of the tap-root cortex of Norway spruce (*Picea abies*) seedlings by the ectomycorrhizal fungus *Laccaria bicolor*. New Phytol 140:723–733.

Kobelt P, Siemens J, Sacristán MD. 2000. Histological characterisation of the incompatible interaction between *Arabidopsis thaliana* and the obligate biotrophic pathogen *Plasmodiophora brassicae*. Mycol Res 104:220–225.

Kowalczyk M, Sandberg G. 2001. Quantitative analysis of Indole-3-acetic acid metabolites of *Arabidopsis*. Plant Physiol 127: 1845–1853.

LeClere S, Tellez R, Rampey RA, Matsuda SPT, Bartel B. 2002. Characterization of a family of IAA-amino acid conjugate hydrolases from Arabidopsis. J Biol Chem 277:20446–20452.

Lindquist S. 1986. The heat shock response. Annu Rev Biochem 55:1151–1191.

Ludwig-Müller J. 1999a. The biosynthesis of auxins. Curr Topics Plant Biol 1:77–88.

Ludwig-Müller J. 1999b. *Plasmodiophora brassicae*, the causal agent of clubroot disease: a review on molecular and biochemical events in pathogenesis. Zeitschr Pflanzenkr Pflanzenschutz 106:109–127.

Ludwig-Müller J. 2000a. Indole-3-butyric acid in plant growth and development. Plant Growth Regul 32:219–230.

Ludwig-Müller J. 2000b. Hormonal balance in plants during colonization by mycorrhizal fungi. In: Douds DD, Kapulnik Y, eds. Arbuscular Mycorrhizas: physiology and function. Dordrecht, The Netherlands: Kluwer Academic Publishers. pp 263–285.

Ludwig-Müller J.. 2003. Peroxidase isoenzymes as markers for the rooting ability of easy-to-root and difficult-to-root *Grevillea* species. In Vitro-Plant 39:377–383.

Ludwig-Müller J, Epstein E. 1991. Occurrence and *in vivo* biosynthesis of indole-3-butyric acid in corn (*Zea mays* L.). Plant Physiol 97:765–770.

Ludwig-Müller J, Epstein E. 1993. Indole-3-butyric acid in *Arabidopsis thaliana*. II. In vivo metabolism. Plant Growth Regul 13:189–195.

Ludwig-Müller J, Epstein E. 1994. Indole-3-butyric acid in *Arabidopsis thaliana*. III. *In vivo* biosynthesis. Plant Growth Regul 14:7–14.

Ludwig-Müller J, Hilgenberg W. 1995. Characterization and partial purification of indole-3-butyric acid synthetase from maize (*Zea mays*). Physiol Plant 94:651–660.

Ludwig-Müller J, Cohen JD. 2002. Identification and quantification of three active auxins in different tissues of *Tropaeolum majus*. Physiol Plant 115:320–329.

Ludwig-Müller J, Hilgenberg W, Epstein E. 1995a. The *in vitro* biosynthesis of indole-3-butyric acid in maize. Phytochemistry 40:61–68.

Ludwig-Müller J, Schubert B, Pieper K. 1995b. Regulation of IBA synthetase by drought stress and abscisic acid. J Exp Bot 46:423–432.

Ludwig-Müller J, Epstein E, Hilgenberg W. 1996. Auxin-conjugate hydrolysis in Chinese cabbage: characterization of an amidohydrolase and its role during clubroot disease. Physiol Plant 97:627–634.

Ludwig-Müller J, Kaldorf M, Sutter EG, Epstein E. 1997a. Indole-3-butyric acid (IBA) is enhanced in young maize (*Zea mays* L.) roots colonized with the arbuscular mycorrhizal fungus *Glomus intraradices*. Plant Sci 125:153–162.

Ludwig-Müller J, Pieper K, Ruppel M, Cohen JD, Epstein E, Kiddle G, Bennett R. 1999. Indole glucosinolate and auxin biosynthesis in *Arabidopsis thaliana* L. glucosinolate mutants and the development of the clubroot disease. Planta 208:409–419.

Ludwig-Müller J, Schubert B, Rademacher W, Hilgenberg W. 2000a. Indole-3-butyric acid biosynthesis in maize is enhanced by cyclohexanedione herbicides. Physiol Plant 110:544–550.

Ludwig-Müller J, Krishna P, Forreiter C. 2000b. A glucosinolate mutant of *Arabidopsis thaliana* is thermosensitive and defective

in cytosolic Hsp90 expression after heat stress. Plant Physiol 123:949–958.

Ludwig-Müller J, Siemens J, Keller I, Schuller A, Nagel W, Parniske M. 2003. Genomic analysis of the clubroot infection of Arabidopsis caused by the parasite *Plasmodiophora brassicae*. Plant Biology, Honolulu, USA.

Mosse B. 1957. Growth and chemical composition of mycorrhizal and non-mycorrhizal apples. Nature 179:922–924.

Müller P, Hilgenberg W. 1986. Isomers of zeatin and zeatin riboside in clubroot tissue: evidence for trans-zeatin biosynthesis by *Plasmodiophora brassicae*. Physiol Plant 66:245–250.

Nelsen CE, Safir GR. 1982. Increased drought tolerance of mycrorrhizal onion plants caused by improved phosphorous nutrition. Planta 154:407–413.

Neuhaus K, Grsic-Rausch S, Sauerteig S, Ludwig-Müller J. 2000. *Arabidopsis* plants transformed with nitrilase 1 or 2 in antisense direction are delayed in clubroot development. J Plant Physiol 156:756–761.

Normanly J, Slovin JP, Cohen JD. 1995. Rethinking auxin biosynthesis and metabolism. Plant Physiol 107:323–329.

Nover L. 1991. Heat shock response. Boca Raton, FL: CRC Press..

Oetiker JH, Aeschbacher G. 1997. Temperature-sensitive plant cells with shunted indole-3-acetic acid conjugation. Plant Physiol 114:1385–1395.

Östin A, Moritz T, Sandberg G. 1992. Liquid chromatography/mass spectrometry of conjugates and oxidative metabolites of indole-3-acetic acid. Biol Mass Spectrom 21:292–298.

Rausch T, Butcher DN, Hilgenberg W. 1983. Indole-3-methylglucosinolate biosynthesis and metabolism in clubroot diseased plants. Physiol Plant 58:93–100.

Rodman JE. 1991. A taxonomic analysis of glucosinolate-producing plants, part I: phenetics. Syst Bot 16:598–618.

Sammuel G, Garret SD. 1945. The infected root-hair count estimating the activity of *Plasmodiophora brassicae* Woron in the soil. Ann Appl Biol 32:96–101.

Schellenbaum L, Berta G, Ravolanirina F, Tisserant B, Gianinazzi S, Fitter AH.. 1991. Influence of endomycorrhizal infection on root morphology in a micro-propagated woody plant species (*Vitis vinifera* L.). Ann Bot 68:135–141.

Schuller A, Ludwig-Müller J. 2002. Isolation of differentially expressed genes involved in clubroot disease. Plant Protect Sci 38special issue 2:483–486.

Searle LM, Chamberlain K, Rausch T, Butcher DN. 1982. The conversion of 3-indolemethylglucosinolate to 3-indoleacetonitrile by myrosinase and its relevance to the clubroot disease of the cruciferae. J Exp Bot 33:935–942.

Siemens J, Nagel M, Ludwig-Müller J, Sacristán MD. 2002. The interaction of *Plasmodiophora brassicae* and *Arabidopsis thaliana*; parameters for disease quantification and screening of mutant lines. J Phytopathol 150:592–605.

Smith SE, Gianinazzi-Pearson V. 1988. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. Annu Rev Plant Physiol Plant Mol Biol 39: 221–244.

Sutter EG, Cohen JD. 1992. Measurement of indolebutyric acid in plant tissues by isotope dilution gas chromatography-mass spectrometry analysis. Plant Physiol 99:1719–1722.

Tam YY, Epstein E, Normanly J. 2000. Characterization of auxin conjugates in *Arabidopsis*. Low steady-state levels of indole-3-acetyl-aspartate, indole-3-acetyl glutamate, and indole-3-acetyl-glucose. Plant Physiol 123:589–596.

Tisserant B, Gianinazzi S, Gianinazzi-Pearson V. 1996. Relationships between lateral root order, arbuscular mycorrhiza development, and the physiological state of the symbiotic fungus in *Platanus acerifolia*. Can J Bot 74:1947–1955.

van der Krieken WM, Breteler H, Visser MHM. 1992. The effect of conversion of indolebutyric acid into indoleacetic acid on root formation. Plant Cell Physiol 33:709–713.

van der Krieken WM, Breteler H, Visser MHM, Mavridou D. 1993. The role of the conversion of IBA into IAA on root generation in apple: introduction of a test system. Plant Cell Rep 12:203–206.

Walker C. 1992. Systematics and taxonomy of the arbuscular endomycorrhizal fungi (Glomales) –a possible way forward. Agronomic 12:887–892.

Walz A, Park S, Slovin JP, Ludwig-Mueller J, Momonoki YS, Cohen JD. 2002. A gene encoding a protein modified by the phytohormone indoleacetic acid. Proc Natl Acad Sci USA 99:1718–1723.

Wenke, T, Ludwig-Muller, T (2002) Genetic and physiological characterization of the glucosinolate deficient *A. thaliana tu8* mutant. Deutsche Botanikertagung, Germany.

Yano K, Yamauchi A, Kono Y. 1996. Localized alteration in lateral root development in roots colonized by an arbuscular mycorrhizal fungus. Mycorrhiza 6:409–415.

Frankenberger, Jr, WT, Arshad, M. (1995) Microbial synthesis of auxins. In: Phytohormones in soils (Frankenberger, WT, Arshad, M, eds.) New York: Marcel Dekker Inc., pp 35–71.