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Improvement of root architecture under abiotic stress through control of auxin homeostasis in Arabidopsis and Brassica crops

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Auxin plays an important role in many aspects of plant development including stress responses. Here we briefly summarize how auxin is involved in salt stress, drought (i.e. mostly osmotic stress), waterlogging and nutrient deficiency in Brassica plants. In addition, some mechanisms to control auxin levels and signaling in relation to root formation (under stress) will be reviewed. Molecular studies are mainly described for the model plant *Arabidopsis thaliana*, but we also like to demonstrate how this knowledge can be transferred to agriculturally important Brassica species, such as *Brassica rapa*, *Brassica napus* and *Brassica campestris*. Moreover, beneficial fungi could play a role in the adaptation response of Brassica roots to abiotic stresses. Therefore, the possible influence of *Piriformospora indica* will also be covered since the growth promoting response of plants colonized by *P. indica* is also linked to plant hormones, among them auxin.

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Introduction

Plant development is dependent on the availability of water and nutrients in a given growth habitat. Severe disbalances in the soil status such as elevated salinity, nutrient deficiencies, and critical fluctuations in water content usually caused by climate changes (drought and waterlogging), determined as abiotic soil stresses or below-ground stresses, affect plant development and crop productivity. Plants have developed different mechanisms to respond to environmental changes by employing plasticity in their growth and development. These molecular mechanisms define a multi-dimensional network of gene regulation, environmental signal sensing, signal transduction, signal responses and phenotype realisation (Yi and Shao 2008). The plant root is directly exposed to below-ground stresses, and responsible for sensing and responding to such unfavorable environmental conditions. The responses of plants to abiotic stress include numerous endogenous signals that coordinate processes within the root tissue, but also between root and subsequent shoot tissues in order to achieve stress adaptation within the whole plant. Therefore, the improvement of the root responses will also result in larger aerial plant parts under abiotic stresses translating eventually into higher yield.

Improvement of root architecture is central for the adaptation of plants to abiotic stress. Among the crucial regulators in controlling these adaptation processes are rather simple indolic compounds called auxins. These hormones are particularly important for root adaptation processes in Brassica crops. Thus, the question arises whether it is possible to obtain better resistance against abiotic stress in Brassica species by co-cultivation with the endophyte *P. indica* since the fungus changes the auxin homeostasis in the roots (Lee et al. 2011; Dong et al. 2013).

The spatiotemporal control of auxin levels is crucial to the development of plants; in particular, to the proliferation of the plant root system. This kind of control cannot be achieved by directed and undirected auxin transport alone, but rather has to include biochemical processes that are known to alter cellular auxin levels. A set of interesting genes specifically contributing to indole-3-acetic acid (IAA) conjugation and deconjugation as well as a number of auxin biosynthesis genes that have been shown to be expressed in roots and to be affected by a number of different stress conditions play important roles in the control of root development. The model system Brassica can adapt to environmental stress conditions by optimizing the root architecture and its communication with the shoot by cocultivation with the root-colonizing endophyte *P. indica*. This adaptation process is mediated by re-adjustment of the auxin homeostasis and that this is mainly caused by local increase in auxin levels (Sirrenberg et al. 2007; Vadassery et al. 2008; Schäfer et al. 2009; Hilbert et al. 2012, 2013; Lee et al. 2011, Dong et al. 2013).

Auxin homeostasis and abiotic stress

What is the exact role of auxin in regulating abiotic stress responses and how can manipulation of the auxin homeostasis generate more abiotic stress tolerant in Brassica plants? *P. indica*-induced adaptation process to drought stress, e.g., results in more resistant plants with >20% increase in biomass and seed production, which is associated with a massive alteration of the root architecture, root-shoot communication and local auxin levels (Peškan-Berghöfer et al. 2004). These

processes are triggered by a low-molecular mass elicitor released by the fungus into the rhizosphere that targets plant auxin metabolism in Brassica crops (Vadassery et al. 2009a; Lee et al. 2011; Dong et al. 2013). Expression profiles of auxin biosynthesis and metabolism genes under stress conditions can be compared with global gene expression profiles obtained under abiotic stress treatments and after *P. indica* colonization in the model plant *Arabidopsis thaliana* and the crop plants *Brassica rapa* and *Brassica napus* by a systems biology approach. Target genes can be identified which could be used as markers for breeding strategies. Differential display identified many *P. indica*-stimulated genes for regulatory proteins in roots (Vahabi et al. 2015). Interesting candidate genes are also regulated by auxin, although exogenous auxin application to Brassica roots does not induces the root hair phenotype.

Root system architecture is mainly determined by the complex interplay of several plant hormones, e.g. auxin, ethylene (ET), and cytokinin. In this network, auxin is elementary to the formation of lateral roots, which largely contribute to the plasticity of the root system. The control of root branching determines the expansion of the root system and, thus, the area of soil penetrated by a plant. Together with the obvious increase in the absorbing-active surface, this is crucial for optimal waterlogging and nutrient uptake via the root system. The potential to take up water and dissolved salts from the rhizosphere essentially decides on the biological fitness of a plant. It might be possible to study these processes by using *P. indica*, which induces a massive stimulation of root hair development, both in Petri dishes and natural soil.

The plasticity of plant development and its potential to adapt to constantly changing environmental conditions imply that the underlying regulatory mechanisms are highly complex. In this respect, the differential distribution of auxin within a given tissue, i.e. the formation of local auxin maxima or gradients, is a particularly important regulatory matter. Auxin gradients have been implicated in numerous developmental processes, for example, pattern formation, embryogenesis, and tropisms. So far, the formation of auxin gradients has been attributed to two different processes, local auxin biosynthesis (Zhao 2010) and directional intercellular auxin transport (Tanaka et al. 2006). Both processes are tightly controlled by a wealth of biotic and abiotic, as well as developmental signals. Hence, the control of cellular auxin formation and its distribution provides a valuable means to efficiently translate internal and external signals into plant growth responses. Basically any plant cell is assumed to have the ability to perceive auxin signals, including root cells.

Auxin in root development

Over the past years, most work has been focused on the elucidation of molecular mechanisms by which the integration of auxin signals in roots is realized. The studies provided evidence for a number of short signal transduction cascades that govern root growth responses by transcriptional reprogramming of responding cells. Herein, they control both priming of pericycle cells to become lateral root founder cells and activation of asymmetrical cell divisions as the initial step of lateral root formation. The pathways involve an auxin specific receptor, an F box protein named TIR1, and several transcriptional repressors of the Aux/IAA family, which are targeted for proteolysis by the receptor complex. The Aux/IAAs orchestrate the activity of a group of transcription factors, mainly from the ARF family (De Rybel et al. 2010; Overvoorde et al. 2010). Alongside these signal transduction and transcription controlling processes, directional auxin transport has been extensively studied over the past couple of years. Members of the PIN-formed (PIN) protein family have been shown to govern auxin efflux in plant as well as in heterologous systems (Grunewald and Friml 2010). However, it has also become increasingly clear that local auxin biosynthesis is crucial to the generation of local auxin maxima. This holds true not only for pattern formation in aerial plant organs, but also for roots, as numerous genetic and pharmacologic studies revealed that auxin can be synthesized not only in leaves and shoot apical meristems, but also in root tissues (Müller et al. 1998; Ljung et al. 2001; Petersson et al. 2009; Hentrich et al. 2013a). Likewise, IAA conjugation has been shown to contribute to the complex regulation of root development (Khan and Stone 2007; Zhang et al. 2007). In general, a sophisticated network of several biochemical reactions including de novo biosynthesis, formation and decomposition of IAA sugar and IAA amino acid conjugates, and translocation processes control cellular auxin levels. Each of these reactions is differentially regulated and can respond to environmental triggers in an appropriate manner.

Auxin conjugates are thought to play important roles as storage forms for the active plant hormone IAA. In its free form, IAA comprises only up to 25 percent of the total amount of IAA, depending on the tissue and the plant species studied. The major forms of IAA conjugates are low molecular weight ester or amide forms, but there is increasing evidence on the occurrence of peptides and proteins modified by IAA (reviewed in Ludwig-Müller 2011). Since the discovery of genes and enzymes involved in synthesis and hydrolysis of auxin conjugates, much knowledge has been gained on the biochemistry and function of these compounds. In most tissues the auxin responses are concentration dependent and different tissues respond in a distinct manner to varying amounts of exogenous auxins (Thimann 1938). Higher auxin concentrations might be often inhibitory, so the optimum endogenous level must be tightly controlled. This tight control of auxin homeostasis is also involved in the regulation of root growth since primary root growth is inhibited by high auxin levels, but lateral root initiation is triggered by local maxima of auxin. For example, mutants in GH3-9, a protein belonging to class II enzymes of auxin conjugate synthetases, displayed a shorter root phenotype and higher sensitivity to auxin-regulated root growth, indicating a role for GH3-9 in root development (Khan and Stone 2007). Another example provides the symbiosis of *P. indica* with an Arabidopsis auxin overproducer, where the fungus promotes growth by converting free auxin into conjugates (Vadassery et al. 2008).

Indications for functions of auxin conjugation in abiotic stress responses come from work with a variety of plant species. Junghans et al. (2006) found an auxin conjugate hydrolase from poplar in salt stressed tissue. Overexpression of PcILL3 in Arabidopsis also rendered these transgenic lines more salt tolerant. During a waterlogging experiment with soybean, a putative auxin conjugate hydrolase was found to be upregulated (Alam et al. 2010). Furthermore, temperature sensitive cells of henbane had altered auxin conjugate levels (Oetiker and Aeschbacher 1997). *GH3* auxin conjugate synthetase genes are also directly involved in stress tolerance. *WES1* (*GH3-5*) for example was also induced by various stress conditions such as cold, drought and heat treatment

as well as by the stress hormones salicylic acid (SA) and abscisic acid (ABA). It is interesting to note that WES1 cannot only adenylate IAA but also SA (Staswick et al. 2005). A *WES1* overproducing line (*wes1-D*) was resistant to abiotic stresses such as drought, freezing and salt, but also high temperatures (Park et al. 2007), whereas a T-DNA insertional mutant showed reduced stress resistance. In addition, stress-responsive genes were up-regulated in the *wes1-D* mutant. Interestingly, *CBF* (*C-repeat/dehydration-responsive element-binding factor*) genes were directly regulated by auxin (repressed by IAA), and the repression was attenuated in the line with higher auxin conjugate formation (Park et al. 2007).

Root architectural alteration by an endophyte

As already insinuated, a potential help for optimizing the root architecture under abiotic stress conditions might provide the symbiotic, root-colonizing endophytic fungus *P. indica*, a basidiomycete of the Sebacinales. This primitive fungus interacts also with the model plant *A. thaliana*. *P. indica* is a cultivable fungus and can grow on synthetic media without a host (Peškan-Berghöfer et al. 2004). *P. indica* promotes nitrate and phosphate uptake and metabolism (Shahollari et al. 2005; Sherameti et al. 2005; Yadav et al. 2010), allows plants to survive under water and salt stress (Sherameti et al. 2008a; Baltruschat et al. 2008; Sun et al. 2010), and stimulates growth, biomass and seed production (Verma et al. 1998; Shahollari et al. 2004, 2005; Sherameti et al. 2005, 2008b; Vadassery et al. 2008; Waller et al. 2005, 2008; Oelmüller et al. 2009). In Arabidopsis and Chinese cabbage (*Brassica rapa*) growth promotion can also be achieved by an exudate component released by the fungus into its cell wall and into the growth medium (Vadassery et al. 2009a; Lee et al. 2011). This and other data support the concept that (an) elicitor released by the fungus is perceived by the host cell, activating a receptor-mediated signalling pathway. Phytohormones and their homeostasis in the roots are crucial for *P. indica* effects in plants (Sirrenberg et al. 2007; Vadassery et al. 2009b; Schäfer et al. 2009; Lee et al. 2011; Dong et al. 2013). The exudate component induces massive root branching, and results in a more than 2-fold increase in the auxin level in the roots (but not in the shoots) of Chinese cabbage seedlings (Lee et al. 2011). Furthermore, the aerial parts of these plants are significantly more resistant to drought (Sun et al. 2010). Since exogenous application of auxin to Chinese cabbage roots cannot replace the beneficial effects of the fungus, characterization of the already identified *P. indica*- and auxin-related targets of *P. indica* in Chinese cabbage roots might contribute substantially to the better understanding of this scenario.

Recent publications have emphasized that a tight control of local auxin contents is highly important for the regulation of root development. This requires sophisticated mechanisms, which control auxin biosynthesis, and the hydrolysis of auxin conjugates in roots during abiotic stress. The beneficial growth-promoting fungus *P. indica* induces stress tolerance and root growth by controlling local auxin levels and auxin related processes, providing a tool to induce the desired phentype, which can be studied with the aim of biotechnological applications. How roots perceive and respond to below-ground abiotic stresses, and how the root architecture is adapted to the novel conditions in terms of development, root-shoot communication and interactions with the biotic environment is still unclear. The response is mediated by adaptation of the auxin homeostasis to change root development in response to abiotic stresses. The perception of the signal by *P. indica* trigger auxin response pathways under normal growth and stress conditions, resulting in enhanced abiotic stress tolerance traits of the colonized plants. In addition, *P. indica* protects the plant systemically under stress using root to shoot communication, which is achieved by taking advantage of the plant's signaling pathways. This generates a strong interaction of the plant roots with the environmental soil. Response to changing environments and adaptation of the roots to these novel conditions requires primarily (local) increase in auxin levels. Changes in auxin homeostasis, i.e. via biosynthesis and release from inactive conjugates might be a direct or indirect consequence of *P. inidca* colonization. *P. indica* confers stress tolerance to Chinese cabbage roots via changes in the auxin homeostasis. Moreover, the fungus utilizes several plant hormone signaling pathways to confer increased tolerance and thus influences the hormonal networks in roots under given stress conditions (Schäfer et al. 2009; Sun et al. 2014).

Four kinds of abiotic stresses impairing plant growth and biomass production play major roles in agriculture: salt stress, drought, waterlogging and nutrient deficiency. In terms of nutrient deficiency, phosphate (P) deficiency studies have demonstrated that limiting P conditions have dramatic impact on root architecture. The effect is governed by the interplay of auxin-dependent cell cycle control, modulation of auxin perception, and auxin accumulation (López-Bucio et al. 2005; Sánchez-Calderón et al. 2005; Pérez-Torres et al. 2008; Miura et al. 2010). Additionally, as outlined above, *P. indica* promotes P uptake (Yadev et al. 2010; Kumar et al. 2011; Molitor et al. 2011; Petersen et al. 2013; Das et al. 2014). This suggests a linkage between the beneficial properties attributed to the symbiosis of the host plant with the endophyte and the influence on the auxin level in the course of fungus infection. However, the selected kinds of stress are causing severe problems in agriculture and reduce crop yields all over the world. It is estimated that considerable losses in crop productivity (more than 50%) are caused by abiotic stresses (Qin et al. 2011); hence, threaten the food security worldwide. throughout Europe. Over 6% of the global land area has estimated to be affected by salinity, 64% by drought, 13% by flooding, and about 57% by extreme temperatures (Munns and Tester 2008; Cramer et al. 2011; Ismail et al. 2014). In the last few years waterlogging became a topic arising considerable public and scientific attention in Central Europe, where large rain falls resulted in rivers overflowed more often than in the past. Members of the Brassicaceae are found more or less all over the world and represent a large number of economically important plant culture crops. Brassica vegetables like cabbages, broccoli, turnip greens and leaf rape, among others, are consumed throughout the world. FAO Statistics (FAOStat 2013) showed that the production of cabbages and other brassicas was 6.3%, and 8% of the total vegetable production of the world, and European Union, respectively, in 2013. The model plant *A. thaliana* can be used to elucidate general mechanisms by functional and genetic analysis.

In conclusion, understanding auxin homeostasis-based networks that govern the outcomes of plant growth responses to a number of different abiotic stress conditions in roots is an important task for the future. The results will be of great significance for studies of crosstalk signaling not only in plant-pathogen but also in plant-stress interactions. The broader merit could result in improved stress resistance

incorporated into commercial lines that is of great and immediate significance for the general public, seed industry, oilseed rape growers and breeders. Ultimately, improved stress tolerance is expected to lead to increased seed yield and biomass production. With the background knowledge of a steady rising demand for agricultural products and limited resources (arable land), this is and will become an even more important matter of concern. Achievement of these goals is possible, as demonstrated by *P. indica* colonization, however our present knowledge about the auxin targets of the fungus prevents the development of biotechnological applicational tools.

How to control auxin signalling, biosynthesis and metabolism

Unlike animals, plants remain fixed in one place absorbing water as well as micro- and macronutrients from their environment. The root system plays a highly important role in this way of life. On the one hand, it serves as the organ of absorption for water and dissolved salts; on the other hand, it is the organ of attachment, anchoring the plant to the ground. As plants have to cope with ever-changing and often adverse environmental conditions, they remained the ability to respond to changes in nutrient and water availability, as well as to answer biotic and abiotic stress cues. In general, optimizing the metabolism or adapting the body plan to the given environmental demands mediates these responses. With respect to the root system architecture, either increasing or decreasing the velocity of root growth and expansion achieves the latter.

Abiotic stress results in a series of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity (Wang et al. 2001). Much abiotic stresses, e.g. drought, salinity, or extreme temperatures, appear to be interconnected and seemingly induce similar cellular damage. As an example, drought and salinity are disclosed primarily as osmotic stress, resulting in the disruption of homeostasis and ion distribution in the cell (Serrano et al. 1999; Zhu 2001). As a consequence, the diverse environmental stress cues often activate similar cell signalling cascades (Shinozaki and Yamaguchi-Shinozaki 2000; Knight and Knight 2001; Zhu 2002) and trigger similar cellular responses, such as the production of stress proteins, up-regulation of anti-oxidants, and accumulation of compatible solutes (Vierling and Kimpel 1992; Zhu et al. 1997; Cushman and Bohnert 2000). Likewise, they induce alteration of plant hormone homeostasis, in particular of ABA and jasmonic acid (JA) (Chaves et al. 2003; Creelman and Mullet 1995; Dombrowski 2003), which, in turn, translates into adaptation of the plant developmental program. Due to the high degree of developmental plasticity, plants are able to respond by completing their life cycle before prevalent stress conditions cause physiological deficits. However, such premature completion of the life cycle to ensure survival of the progeny leads to a significant loss in plant productivity. Experiments employing the endophytic fungus *P. indica* provided evidence for a significantly increased stress tolerance and productivity of infected plants, indicating that the reign of growth inhibiting plant hormones can be broken. It is becoming increasingly clear that the fungus produces its beneficial impact by manipulating auxin homeostasis, but the underlying molecular mechanisms have yet to be disclosed.

Research on abiotic stress involved the screening for mutants directly affected in individual stress components such as salt or drought or waterlogging, etc. By this approach individual components of a particular stress pathway have been identified among them transporters for sodium (Qiu et al. 2004) or members of signal transduction chains (Gong et al. 2002). However, the root architecture has been neglected as a common target in these studies. The identification of a single component transferring stress tolerance to only one stress is the result of these studies. On the other hand, improving the root architecture as a whole in response to a variety of stresses and stress combinations will result in plants tolerant to more than one abiotic stress situation. To improve the root system by using natural compounds, i.e. auxins will be important for the understanding root development. So far, the involvement of auxins as signaling molecules in plant stress responses is only poorly exploited. However, there are already a few lines of evidence from genomic studies that indicate a contribution of auxin in this process (Sreenivasulu et al. 2007; Wang et al. 2010; Dombrecht et al. 2007; Hentrich et al. 2013a). In this context it is important to analyse also hormonal crosstalk between stress and growth-related hormones as one aspect to identify combinations increasing stress tolerance.

The initiation of lateral roots is of particular importance for the absorption efficiency of a plant, as it directly determines the active surface of the root system. Plants produce new organs, such as lateral roots, primarily postembryonically. The formation of these new lateral organs proceed from rudimentary formed founder cells that follow a precise pattern, which guarantees an even and optimal spacing of the lateral roots that contributes to the functionality of the newly formed plant organs. Although several different compounds are known as plant hormones and to contribute to plant stress responses, either acting individually or in crosstalk with other phytohormones (Davies 2004), plant shape is largely controlled by auxins (Vanneste and Friml 2009). In roots, both priming of the lateral root founder cells as well as initiation of the asymmetrical dividing of these pericycle cells and their development is predominantly governed by auxin, and a number of AUXIN RESPONSE FACTORS (ARFs) and AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) proteins that act as inhibitors of the ARFs.

It has been reported that the control of lateral root founder cell identity is facilitated by an IAA28-dependent auxin signalling module in the basal meristem region of the primary root that regulates the expression of the transcription factor GATA23 that seemingly plays a key role in specifying pericycle cells to become lateral root founder cells prior to lateral root initiation (De Rybel et al. 2010). Lateral root initiation itself, is largely maintained by the SOLITARY ROOT (SLR)/IAA14-ARF7-ARF19 auxin response system that is necessary for proper activation of the basic cell cycle machinery and for the control of the initial, asymmetric pericycle cell divisions (Fukaki et al. 2002; Vanneste et al. 2005; Fukaki and Tasaka 2009).

As can be taken from these few examples, the dynamic and spatiotemporal presence of IAA as well as the ability to perceive and integrate this signal has to be tightly controlled, in order to enable appropriate growth of the root system. Extremely simplified, it can be assumed that low IAA contents translate into a repression of lateral root formation, while elevated levels of IAA result in an increased number of lateral root founder cells and, hence, an increased number of lateral roots. This summary is not entirely correct, though, since it is well-known that, especially in roots, high IAA levels can have growth inhibitory effects (Thimann 1938; Ivanchenko et al. 2010). Nevertheless, it provokes the crucial question on how cellular auxin levels are controlled. One major aspect

in the generation of local auxin maxima is surely the cell-to-cell transport of IAA by polar localized auxin exporters of the PIN-FORMED (PIN) family and some evenly distributed ATP-binding cassette subfamily B (ABCB)-type transporters of the multidrug resistant/phosphoglycoprotein (ABCB/MDR/PGP) protein family. Accompanied are these transporters by auxin importers of the AUX1/LAX family (Petrasek and Friml 2009). However, several recent studies have led to the discovery that also local auxin biosynthesis is crucial to many developmental processes including pattern formation, gametogenesis, embryogenesis, seedling growth, and flower development (Zhao 2010). Moreover, it has been suggested that the release of free auxin from auxin-conjugates additionally contributes to various developmental aspects (Ludwig-Müller 2011; see Figure 1).

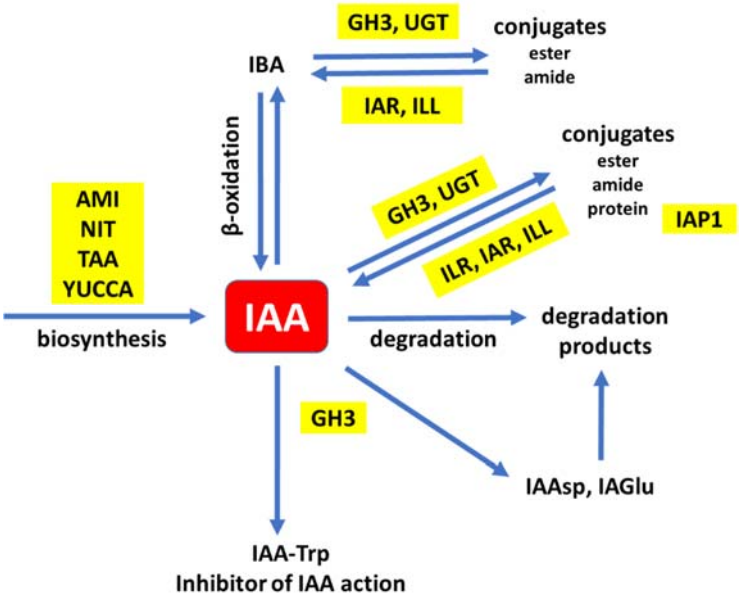

Figure 1: Pathways to regulate auxin homeostasis by biosynthesis and conjugation.

Regulation of auxin

Despite the mounting knowledge of the processes governed by auxins and of auxin transport, the biosynthesis of IAA is still uncertain. IAA biosynthesis is suggested to proceed via a number of different metabolic routes. Up to date, only one of the proposed pathways is fully disclosed with respect to the catalyzed reaction steps and the enzymes involved. However, it is proposed that the different pathways act either in parallel or redundantly to one another, in a developmentally regulated manner (Zhao 2010). In *A. thaliana*, comprehensive mutant analyses have implicated two major biosynthetic pathways in auxin biosynthesis; the seemingly prevalent IAA-biosynthetic pathway that takes a tryptophan aminotransferase (*TAA1*/*SAV3*) dependent route (Stepanova et al. 2008; Tao et al. 2008), which proceeds via a final *YUCCA* (*YUC*) monooxygenase-like family-dependent reaction step (Zhao et al. 2001; Mashiguchi et al. 2011; Won et al. 2011; Stepanova et al. 2011), and the *CYP79B2/B3* cytochrome P450 monooxygenase family-dependent pathway (Zhao et al. 2002; Sugawara et al. 2009). The lack of *CYP79B2/B3* homologs in genera other than Arabidopsis and very few closely related species most likely restricts the latter pathway to the Brassicaceae family and contradict a widespread distribution of this route within the plant kingdom (Quittenden et al. 2009; Sugawara et al. 2009). Besides the above-mentioned biosynthetic pathways, several other publications reported the operation of an IAM hydrolase-dependent pathway to occur *in planta* (Pollmann et al. 2003; Arai et al. 2004; Nemoto et al. 2009). Especially the identification of IAM as an endogenous compound in Arabidopsis (Pollmann et al. 2002), and the subsequent isolation and characterization of an IAM hydrolase (AMI1) from this species, but also several other mono and dicot species, implicated the functionality of such a pathway in plants (Pollmann et al. 2003, 2006; Neu et al. 2007; Sánchez-Parra et al. 2014).

Various features in root architecture such as increase/decrease of primary root length, number of lateral roots and root hairs all are connected to root surface which affects viability of the upper part of the plant. Auxins play a major role in root growth and development, and thus also in abiotic stress tolerance. In additon to auxin several other plant hormones are involved in the control of root development, such as cytokinin, JAs, and ET.

Previous research characterized the first described IAM hydrolase from plants, referred to as *AMIDASE1* (*AMI1*) (Pollmann et al. 2003, 2006; Neu et al. 2007). The Arabidopsis AMI1 shows high homology to bacterial iaaH auxin biosynthesis enzymes from plant pathogens, such as Pseudomonas and Agrobacterium. Metabolic and genetic studies have underlined the contribution of AMI1 to auxin biosynthesis in planta, too (Lehmann et al. in revision). The induced ectopic expression of *AMI1* results in auxin-related mutant phenotypes, i.e. short primary roots, reduced growth, and curled leaf shapes. Moreover, the overexpressors have increased IAM hydrolase activities, and elevated endogenous IAA contents. Promoter reporter lines as well as qRT-PCR experiments revealed strong *AMI1* expression during seedling development and in proliferating tissues (Pollmann et al. 2006; Hoffmann et al. 2010; Lehmann et al. in revision), resembling the expression pattern of other auxin biosynthesis genes, such as *YUC4*, *TAA1*, and *CYP79B2*, and of the synthetic auxin reporter *DR5*::*GUS* (Ulmasov et al. 1997; Mikkelsen et al. 2000; Cheng et al. 2007; Tao et al. 2008). Besides several biotic cues, numerous abiotic stimuli have been observed to regulate the expression of *AMI1.* In particular, salt stress has been reported to significantly up-regulate the expression of *AMI1*, whereas high sugar contents have been shown to suppress *AMI1* transcription. The latter implies crosstalk between auxin formation and sugar signaling networks, whereby plant growth is presumably linked with photosynthesis and carbon fixation (Lehmann et al. 2010).

In contrast to the results obtained in the gain-of-function experiments, the analysis of an *AMI1* T-DNA insertion mutant disclosed reduced IAM hydrolase activities and a significantly reduced endogenous IAA levels. Interestingly, the in-depth analysis of the root architecture of the three *ami1* knockout line under control and salt stress conditions provided strong evidence for a function of *AMI1* as a suppressor of root branching and of the expansion of the root system. This function became even more pronounced when plantlets were geminated under salt stress conditions. While the wild type responded with a slight reduction of root branching to high salinity in the media, the *ami1* null mutant answered to this type of abiotic stress with a reasonable induction of root branching. So far, it is not entirely clear how AMI1-governed

auxin production can negatively affect lateral root formation and the expansion of the root system. Currently, we favour the working hypothesis that this way of auxin formation counteracts local auxin maxima in such way that it reduces the steepness of the auxin gradients by the production of IAA in surrounding tissues. Consequently, this may override local auxin signals necessary for the initiation of lateral root formation, and translate in an arrest of lateral root development. Alternatively, AMI1-mediated auxin biosynthesis might result in general auxin overproduction, which may have a global inhibitory effect on root proliferation. However, it is yet too early to decide which molecular mechanism is responsible for the suppressive function of AMI1, as insight into the dynamic expression of *AMI1* during the course of lateral root formation is yet to be provided.

Some other previous studies unraveled substantial crosstalk between oxylipins and auxin. This is remarkable in such as these two plant hormones usually have antagonistic effects. However, it appeared that the expression of one *YUCCA* (*YUC*) gene family member, *YUC9*, is strongly induced by JA and coronatine, a bacterial phytotoxin that mimicks the physiologically active form of JA, JA-Ile. By contrast, the bioactive JA precursor, 12-oxo-octadecatrienic acid (OPDA), suppresses *YUC9* transcription. Mutant analysis, employing the JA receptor mutant *coi1*, reveaved that JA-mediated auxin biosynthesis proceeds via the COI1 signalling pathway. Metabolic studies on dissected organs of wild type and *yuc* mutant lines provided several lines of evidence for a partially redundant system in which *YUC9* acts together with its close homolog, *YUC8*. However, *YUC8* shows only a weak and late response to JA, but is capable of partially compensating for a loss of *YUC9*, as in the *yuc9* background *YUC8* shows an induced responsiveness to JA. Thorough genetic and metabolic analyses indicated that both genes contribute to auxin biosynthesis in planta. Compared to the wild type, *yuc8* and *yuc9* knockouts have shorter primary roots, hypocotyls, and petioles. Moreover, especially the *yuc9* mutants show an aberrant root branching phenotype. Noteably, a loss of both loci, *YUC8* and *YUC9*, translates into a nearly complete lack of IAA production in response to the administration of JA (Hentrich et al. 2013a). Interestingly, JA has additionally been reported to induce the expression of several IAA-amino acid hydrolases, i.e. *IAR3*, *ILR1*, *ILL3*, and *ILL5* (Taki et al. 2005; Salopek-Sondi et al. 2013). The corresponding enzymes could, in turn, be responsible for the remaining IAA formation in the *yuc8*/*yuc9* double-knockout line, thereby providing a functional connection between auxin de novo biosynthesis and auxin release from diverse storage pools. However, these results are in agreement with promoter reporter studies that disclosed expression pattern for both *YUC8* and *YUC9* that imply a participation in lateral root development. Apparently, *YUC9*, but not *YUC8*, is downstream of the repressor protein SOLITARY ROOT/IAA14, as it is clearly up-regulated in the *slr1* null mutant background. Intriguingly, *YUC9* is seemingly not controlled by ARF7/ARF19, the transcription factors mainly affected by SLR/IAA14, since *YUC9* is not significantly repressed in the *arf7/arf19* double-knockout line. However, with respect to the expression pattern, it is stricingly clear that *YUC9* goes ahead of *YUC8* in the course of lateral root formation. *YUC9* expression gets visible at stage II to III of primordia formation in the central cylinder of the root, directly beneath proliferating lateral root primordia. The expression level increases with the onset of lateral root growth but stays restricted to the central cylinder of the primary root. *YUC8* expression, however, lacks behind showing first detectable signals clearly later than stage five at the central base of the newly formed lateral root. Later on, *YUC8* expression stays restricted to the lateral root and increases within the central cylinder of the newly formed organ.

JAs are generally considered to be basic plant stress hormones produced after herbivore or pathogen attack. Nonetheless, there is also a wealth of data providing evidence for a contribution of JA in controlling both developmental processes and plant responses to water deficit, salt stress, and heavy metal stress (Creelman and Mullet 1995; Dombrowski 2003; Maksymiec 2007). It seems evident that plants use JA as a bridge to link plant stress and growth responses. In this framework, the JA-induced and *YUC8*- and *YUC9*-mediated auxin biosynthesis is suggested to result in local IAA overproduction, which leads to two different outcomes: i) high IAA doses repress plant growth, thereby assisting the general growth inhibiting influence of JA; ii) high IAA contents are known to induce ET biosynthesis. ET is known to stimulate the formation of sclerenchymatic tissue and to promote secondary plant growth. Both of these effects can be observed in respective *YUC8* and *YUC9* gain-of-function mutants (Hentrich et al. 2013a, b).

Taken together, *AMI1* and the two *YUCCA* genes, *YUC8* and *YUC9*, from Arabidopsis, are likely involved in regulating lateral root development. Publicly available microarray data point towards a differential regulation of the three candidate genes upon various abiotic stress conditions, including salt-, drought-, and osmotic stress. Furthermore, several types of nutrient stresses, such as nitrogen, phosphorous, sulfur, potassium, or iron deficiency or depletion have been shown to transcriptionally control target gene expression. Possibly even more importantly, the three target genes show very broad distribution in the plant kingdom, implying an important and perhaps basic function. Currently, there are 47 identified AMI1 homologous proteins derived from 38 different species, covering both mono- and dicot genera (Sánchez-Parra et al. 2014). Like AMI1 homologs, YUC-like proteins have been identified from various plant species, including the relevant crop plants maize, rice, and tomato. As *Brassica* crops, such as *Brassica rapa* and *Brassica napus*, are phylogenetically closely related with *A. thaliana* the identification and functional characterization of pendants to *AMI1*, *YUC8*, and *YUC9* in the *Brassica* crops is not expected to be a major obstacle. Another remarkable aspect with respect to the three selected target genes is that a loss of neither of the genes causes lethality, which makes them to ideal candidates for the generation of transgenic plant lines or for the development of targeted breeding programs that yield in elite germplasms with improved traits.

IAA is found mostly in its conjugated form (approximately 95%) in all the seed plants studied so far, thus suggesting that reversible conjugation is, in addition to auxin biosynthesis, another critical mechanism to regulate IAA availability (Sztein et al. 1995). Different plant species have distinct profiles of auxin conjugates; monocots preferably accumulate ester conjugates, whereas dicots synthesize mostly amide conjugates (reviewed by Bajguz and Piotrowska 2009). Since 1955, when IAA-Asp was detected as a first amide conjugate in pea seedlings, different amino acids (see for review Woodward and Bartel 2005; Ludwig-Müller et al. 2009; Penčik et al. 2009) and peptide (Walz et al. 2002, 2008; Seidel et al. 2006) conjugates of IAA were reported to be identified in different plants. It is generally postulated that IAA conjugated to Asp and Glu is an irreversible catabolite being important for auxin detoxification, while other

amid conjugates are suggested to participate in storage and hormone homeostasis as a slow-releasing source of free IAA (Hangarter and Good 1981). Amide conjugates of the long-chain auxin, 4-(indol-3-yl)butyric acid (IBA) (particularly IBA-Asp) were reported to be found in a few plants such as pea tissue (Nordström et al. 1991), and petunia cell suspension culture (Epstein and Ludwig-Müller 1993) after feeding with IBA, and in maize roots during arbuscular mycorrhiza formation (Fitze et al. 2005). In the view of application, exogenous IBA conjugates have been successfully used for the rooting of cuttings (Epstein and Wiesman 1987; Wiesman et al. 1989; Mihaljević and Salopek-Sondi 2012), although mechanism of root initiation has remained unclear.

The conjugate IAA-Trp has a different function from other auxin conjugates, i.e. it is not a storage compound (Staswick 2009). IAA-Trp caused agravitropic root growth in seedlings, while Trp alone did not. In addition, IAA-Trp nearly eliminated seedling root inhibition caused by high concentrations of IAA and inhibited IAA-dependent stimulation of lateral root growth (Staswick 2009). These results showed that IAA-Trp constitute a previously unrecognized mechanism to regulate auxin action and could be therefore an important compound in controlling root architecture.

It has been shown that reversible auxin conjugation may play an important role in plant adaptation to abiotic stress. In poplar trees, the auxin content was found to decline during stress conditions while auxin conjugates were increased (Junghans et al. 2006; Popko et al. 2010), suggesting that diminished auxin content may be a factor that adapts growth (generally reduce) during adverse environmental conditions. Furthermore, perturbation in auxin profile such as elevated level of long chain auxin IBA and its sugar conjugate IBA-Glc in Arabidopsis transgenic plants ectopically expressed UDP-glucosyltransferase (UGT74E2) has been shown to improve survival during drought and salt stress significantly (Tognetti et al. 2010). A cascade of signals caused by stress conditions seems to mediate auxin profile and level by influencing expression and activity of enzymes responsible for reversible auxin conjugation, such as auxin conjugate synthases (GH3), UDP-glucosyltransferases (UGTs), and auxin amidohydrolases (IAR and ILL). Thus various environmental stresses, including high salinity and osmotic stress, are shown to induce *GH3* genes and overproduce GH3 enzymes in Arabidopsis (Park et al. 2007). Furthermore, activation of GH3 promoter has been found in poplar under salt stress by using *pGH3::GUS* as an auxin-responsive reporter (Teichman et al. 2008). Tognetti et al. (2010) showed that UDP-glucosyltransferase (UGT74E2), which preferentially glucosylates IBA as a substrate was strongly upregulated during drought and salt stress. The mechanism of regulation has appeared to be mediated by H_2O_2, usually released during stress conditions. On the other side, Arabidopsis plants transformed with auxin-amidohydrolase gene *ILL3* from poplar were more resistant to salt stress than the wild-type plants (Junghans et al. 2006). Based on proteome analysis, auxin-amidohydrolase was also reported as a novel protein identified in soybean root in response to waterlogging (Alam et al. 2010). Despite a few above-mentioned evidences, a reversible auxin conjugation as a mechanism of abiotic stress adaptation is mostly unclear, particularly in crop plants.

The enzymology involved in the metabolism of amide conjugates is just beginning to be understood. The biosynthesis of amide conjugates is catalyzed by a subset of proteins from the so-called GH3 family and appeared to include auxins activated by adenylation as critical intermediates (Staswick et al. 2002, 2005). The family of *GH3* genes in Arabidopsis consists of 19 family members (Staswick et al. 2005), of which at least seven are able to catalyze the synthesis of IAA amide conjugates. These belong to the so-called group II genes (Staswick et al. 2005). The first member of the auxin inducible *GH3* gene family was isolated from soybean (Hagen and Guilfoyle 1985). Up to now GH3 family members involved most likely in IAA conjugation have been reported from a variety of plant species (for more details see the review on GH3s by Wang et al. 2008), among them the moss *Physcomitrella patens* (Bierfreund et al. 2004; Ludwig-Müller et al. 2009), tobacco (Roux and Perrot-Rechenmann 1997), rice (Jain et al. 2006), and pungent pepper (Liu et al. 2005), the latter regulated by auxin and ET. Despite this, only for a few plant species, in addition to Arabidopsis, the activity of GH3 proteins as adenylating enzymes has been demonstrated, e.g. for *P. patens* (Ludwig-Müller et al. 2009), and rice (Chen et al. 2009). In addition, the GH3 protein family has been implied in the stress response and stress tolerance in Arabidopsis (Park et al. 2007), poplar (Teichmann et al. 2008), sorghum (Wang et al. 2010), rice (Du et al. 2012), and apple (Yuan et al. 2013).

The hydrolysis of IAA amide conjugates results in free auxins and is mediated by auxin amidohydrolases, metalloenzymes belonging to the M20 family of peptidases. Several such hydrolases (named IAR and ILL) were first cloned from *A. thaliana* and tested for activity towards conjugates of the most common auxin, IAA (Bartel and Fink 1995; Davies et al. 1999; Lasswell et al. 2000; LeClere et al. 2002; Rampey et al. 2004). Each of these enzymes shows different but overlapping substrate specificity. Auxin conjugate hydrolases were then isolated and partially characterized from *Arabidopsis suecica* (sILR1), a close relative of *A. thaliana* (Campanella et al. 2003a, b). Some of auxin conjugate hydrolases from the legume *Medicago truncatula* displayed activity towards IAA-aspartate (Campanella et al. 2008), which has earlier been described only as substrate for bacterial IAA conjugate hydrolases (Chou et al. 1998). An ortholog of the *A. thaliana* IAR3 from the monocot species wheat (*Triticum aestivum*) (TaIAR3) showed substrate specificity for longer side chain auxins such as amino acid conjugates with indole-3-butyric acid (IBA) and indole-3-propionic acid (IPA) (Campanella et al. 2004). Furthermore, a family of auxin conjugate hydrolases from Chinese cabbage also showed substrates preferences towards amino acid conjugates in the first place with IPA, then IBA (Savić et al. 2009). In addition, recent theoretical study confirmed that alanine conjugates of IBA and IPA make stronger interactions with the binding site of BrILL2 in comparison to IAA (Šimunović et al. 2011). Also, these hydrolases were differentially regulated during the interaction of Chinese cabbage with the obligate biotrophic pathogen *Plasmodiophora brassicae* (Schuller and Ludwig-Müller 2006). Long-chain auxin IBA appears to be stored in a manner similar to that of IAA in a form conjugated to amino acids, allowing its slow hydrolysis and release. IPA has also auxin activity, but its occurrence as an endogenous auxin is still questionable. It has been found so far in roots of Arabidopsis upon treatment with SA (Walker et al. 2003), while IBA was shown to be elevated in the conditions of drought and high salinity (Tognetti et al. 2010). This is in accordance with early work from Ludwig-Müller et al. (1995), showing that IBA synthesis was drought and salt inducible. These preliminary findings may imply the involvement of long-chain auxins in stress responses. Despite the isolation and characterization of numerous auxin amidohydrolases form different plants to date, the reaction mechanisms as well as

regulation of auxin amidohydrolases on the gene expression level and protein activity level are still unclear. The first x-ray structure of the ILL2 enzyme from Arabidopsis has been reported as an apoenzyme (Bitto et al. 2009), still missing details about substrate binding site and amino acid residues important for substrate specificity. Based on modeling a potential substrate binding cleft has been proposed for the Arabidopsis enzyme (AtILL2) (Bitto et al. 2009), as well as the Chinese cabbage enzyme (BrILL2), in which additionally several substrate binding modes have been predicted for the preferred substrate IPA-Ala (Savić et al. 2009; Smolko et al. under review).

Conclusions and perspectives – *P. indica* may help to understand the role of auxin in mutualistic interaction

Studies on the interaction of the symbiotic, root-colonizing endophytic fungus *P. indica* with the model plant Arabidopsis showed general growth-promoting effects (Peškan-Berghöfer 2004; Lee et al. 2011; Lahrmann et al. 2013). *P. indica*-insensitive mutants are also insensitive to the exudate fraction (Vadassery et al. 2009a; Lee et al. 2011; Johnson et al. 2014a, b). This and other data support the concept that (an) elicitor released by the fungus is perceived by the host cell and activates a receptor-mediated signalling pathway. Consistent with this hypothesis, an atypical receptor-like kinase with leucine-rich repeats is required for the interaction (Shahollari et al. 2007) and a rapid increase in the intercellular calcium concentration and of the second messenger phospholipid phosphatidic acid represent early signalling events (less than 2 minutes) in the root cells (Vadassery et al. 2009a; Camehl et al. 2010). Mutations preventing the increase in the intracellular Ca^{2+} elevation in root cells or the accumulation of phosphatidic acid in response to the exudate of *P. indica* completely prevent the benefits for Arabidopsis plants (Camehl et al. 2010). Phytohormones and their homeostasis in the roots are crucial for *P. indica* effects in plants (Sirrenberg et al. 2007; Vadassery et al. 2008; Schäfer et al. 2009). *P. indica* exudate component also stimulates growth of Chinese cabbage plants. Much work has been performed to understand the role of auxin in the beneficial interaction between *P. indica* and other plant species. In Arabidopsis, growth promotion is achieved without an overall upregulation of the auxin level in roots. However, the draft phenotype of the auxin-overproducer *sur1* is entirely rescued by the fungus by converting free auxin into conjugates (Vadassery et al. 2008). The *P. indica*-induced growth promotion in Chinese cabbage is much stronger than in Arabidopsis, resulting in massive root hair development (Lee et al. 2011). Interestingly, the benefits for the plant do not require a living fungus, but can also be triggered by a low molecular mass exudate component released from the fungus into the fungal cell wall and growth medium. In Chinese cabbage roots this component induces massive root branching, and a more than 2-fold increase in the auxin level in the roots (but not in the shoots) of Chinese cabbage seedlings (Lee et al. 2011), and - after transfer to soil - results in plants, which produce 23% more seeds. Furthermore, the aerial parts of these plants are significantly more resistant to drought (Sun et al. 2010): the adult plants require 440 ml less water for seed production in the greenhouse (control plants require 5100 ml water), and are more resistant against infections by *Alternaria brasssicae* (Johnson et al. 2014a, b). The fungus confers stress tolerance by strongly reducing endogenous and stress-induced reactive oxygen species in Arabidopsis (Matsuo et al. 2015). Since the exudate component of *P. indica* does not contain auxin (Lee et al. 2011), but triggers the benefits in Chinese cabbage plants via the stimulation of auxin homeostasis in the roots, and since exogenous application of auxin to Chinese cabbage roots cannot replace the beneficial effects of the fungus, characterization of auxin-related target genes in Chinese cabbage roots, which are upregulated by the fungus might contribute substantially to the better understanding of this scenario. Overexpression of the *B. rapa* AUX1 IAA influx carrier in Arabidopsis resulted in larger roots even without *P. indica* colonization (Lee et al. 2011). AUX1 was found to be upregulated by *P. indica* in Chinese cabbage (Lee et al. 2011; Dong et al. 2013). This shows that auxin-related genes identified in a transcriptome analysis can serve as targets for improving the root system.

The processes involved in auxin signalling in the root under stress conditions are very complex and cannot be tackled by analysing single or only a few components of a system. A systems biology approach deals with the biological problems in the most general way possible with the help of both advance molecular techniques and mathematical modelling. Technologies available for gene expression profiling allow simultaneous analysis of all genes in the organism. However, this is linked with collection of immense amounts of data that have strong dimensionality problem, representing a challenge both to biologists as well as to statisticians. It was shown that a data analysis workflow where only the intersection of differentially expressed genes list obtained using different preprocessing methods (background correction, normalization, greatly improves the robustness of the obtained results (Rotter et al. 2007). Another implication of huge datasets is their reporting and sharing and FAIR principles (Findable, Accessible, Interoperable and Reusable; Starr et al. 2015). For easier sharing of information and possible re-analyses, data must be stored in permanent public databases (e.g. GEO, ArrayExpress, CIBEX), following adequate MIBBI standard (Taylor et al. 2008) and applying FAIR principles of data sharing. Appropriate 'in-house' organization of transcriptome data is an excellent starting point for designing experiments, generating hypotheses or conceptualizing as well as for cross-species comparison (Mochida and Shinozaki 2010). Another important point is efficient translation of knowledge between model species and crop species which is enabled in GOMapMan Application (Ramšak et al. 2014). Besides statistical analyses, pathway analysis (gene set enrichment methods), data integration and data visualization in the context of biological pathways (such as MapMan) can contribute significantly to the relevant biological interpretation (Rotter et al. 2007, 2009). Finally, a systems biology approach can, through integration of several data sources, lead to construction of regulatory network models of the studied process (Breitling 2010). Those approaches might contribute substantially to a better understanding of the role of the auxin homeostasis under stress.

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