Publikationen - Stoffwechsel- und Zellbiologie

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Eysholdt-Derzsó, E.; Hause, B.; Sauter, M.; Schmidt-Schippers, R. R.; Hypoxia reshapes Arabidopsis root architecture by integrating ERF-VII factor response and abscisic acid homoeostasis *Plant Cell Environ.* (2024) DOI: 10.1111/pce.14914

Abstract
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Oxygen limitation (hypoxia), arising as a key stress factor due to flooding, negatively affects plant development. Consequently, maintaining root growth under such stress is crucial for plant survival, yet we know little about the root system\'s adaptions to low-oxygen conditions and its regulation by phytohormones. In this study, we examine the impact of hypoxia and, herein, the regulatory role of group VII ETHYLENE-RESPONSE FACTOR (ERFVII) transcription factors on root growth in Arabidopsis. We found lateral root (LR) elongation to be actively maintained by hypoxia via ERFVII factors, as erfVII seedlings possess hypersensitivity towards hypoxia regarding their LR growth. Pharmacological inhibition of abscisic acid (ABA) biosynthesis revealed ERFVII-driven counteraction of hypoxia-induced inhibition of LR formation in an ABA-dependent manner. However, postemergence LR growth under hypoxia mediated by ERFVIIs was independent of ABA. In roots, ERFVIIs mediate, among others, the induction of ABA-degrading ABA 8′-hydroxylases CYP707A1 expression. RAP2.12 could activate the pCYC707A1:LUC reporter gene, indicating, combined with single mutant analyses, that this transcription factor regulates ABA levels through corresponding transcript upregulation. Collectively, hypoxia-induced adaptation of the Arabidopsis root system is shaped by developmental reprogramming, whereby ERFVII-dependent promotion of LR emergence, but not elongation, is partly executed through regulation of ABA degradation.

Schreiber, T.; Prange, A.; Schäfer, P.; Iwen, T.; Grützner, R.; Marillonnet, S.; Lepage, A.; Javelle, M.; Paul, W.; Tissier, A.; Efficient scar-free knock-ins of several kilobases in plants by engineered CRISPR/Cas endonucleases *Mol. Plant* (2024) DOI: 10.1016/j.molp.2024.03.013

Abstract
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In plants and mammals, non-homologous end-joining is the dominant pathway to repair DNA double strand breaks, making it challenging to generate knock-in events. We identified two groups of exonucleases from the Herpes Virus and the bacteriophage T7 families that conferred an up to 38-fold increase in HDR frequencies when fused to Cas9/Cas12a in a Tobacco mosaic virus-based transient assay in Nicotiana benthamiana. We achieved precise and scar-free insertion of several kilobases of DNA both in transient and stable transformation systems. In Arabidopsis thaliana, fusion of Cas9 to a Herpes Virus family exonuclease leads to 10-fold higher frequencies of knock-ins in the first generation of transformants. In addition, we demonstrate stable and heritable knock-ins of in wheat in 1% of the primary transformants. Our results open perspectives for the routine production of heritable knock-in and gene replacement events in plants.

Frey, M.; Vahabi, K.; Cankar, K.; Lackus, N. D.; Padilla-Gonzalez, F.; Ro, D.-K.; Rieseberg, L.; Spring, O.; Tissier, A.; Sesquiterpene lactones – insights into biosynthesis, regulation and signalling roles *Crit. Rev. Plant Sci.* 1-27, (2024) DOI: 10.1080/07352689.2024.2307240

Abstract

Internet
RIS
BibTeX

Sesquiterpene lactones (STLs) are bitter tasting plant specialized metabolites derived from farnesyl pyrophosphate (FPP) that contain a characteristic lactone ring. STLs can be found in many plant families that are distantly related to each other and outside the plant kingdom. They are especially prevalent in the plant families Apiaceae and Asteraceae, the latter being one of the largest plant families besides the Orchidaceae. The STL diversity is especially large in the Asteraceae, which made them an ideal object for chemosystematic studies in these species. Many STLs show a high bioactivity, for example as protective compounds against herbivory. STLs are also relevant for pharmaceutical applications, such as the treatment of malaria with artemisinin. Recent findings have dramatically changed our knowledge about the biosynthesis of STLs, as well as their developmental, spatial, and environmental regulation. This review intents to update the currently achieved progress in these aspects. With the advancement of genome editing tools such as CRISPR/Cas and the rapid acceleration of the speed of genome sequencing, even deeper insights into the biosynthesis, regulation, and enzyme evolution of STL can be expected in the future. Apart from their role as protective compounds, there may be a more subtle role of STL in regulatory processes of plants that will be discussed as well.

Wasternack, C.; Hause, B.; BFP1: One of 700 Arabidopsis F-box proteins mediates degradation of JA oxidases to promote plant immunity *Mol. Plant* **17**, 375-376, (2024) DOI: 10.1016/j.molp.2024.02.008

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Launhardt, L.; Uhlenberg, J.; Stellmach, H.; Schomburg, M.; Hause, B.; Heilmann, I.; Heilmann, M.; Association of the Arabidopsis oleoyl Δ12-desaturase FAD2 with pre-cis-Golgi stacks at endoplasmic reticulum-Golgi-exit sites *Plant J.* **117**, 242-263, (2024) DOI: 10.1111/tpj.16492

Abstract
Internet
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The unsaturation of phospholipids influences the function of membranes. In Arabidopsis thaliana, the oleoyl Δ12-desaturase FAD2 converts oleic (18:1Δ9) to linoleic acid (18:2Δ9,12) and influences phospholipid unsaturation in different cellular membranes. Despite its importance, the precise localization of Arabidopsis FAD2 has not been unambiguously described. As FAD2 is thought to modify phospholipid-associated fatty acids at the endoplasmic reticulum (ER), from where unsaturates are distributed to other cellular sites, we

hypothesized that FAD2 locates to ER subdomains enabling trafficking of lipid intermediates through the secretory pathway. Fluorescent FAD2 fusions used to test this hypothesis were first assessed for functionality by heterologous expression in yeast (Saccharomyces cerevisiae), and in planta by Arabidopsis fad2 mutant rescue upon ectopic expression from an intrinsic FAD2 promoter fragment. Light sheet fluorescence, laser scanning confocal or spinning disc microscopy of roots, leaves, or mesophyll protoplasts showed the functional fluorescence-tagged FAD2 variants in flattened donut-shaped structures of ~0.5–1 µm diameter, in a pattern not resembling mere ER association. High-resolution imaging of coexpressed organellar markers showed fluorescence-tagged FAD2 in a ring-shaped pattern surrounding ER-proximal Golgi particles, colocalizing with pre-cis-Golgi markers. This localization required the unusual C-terminal retention signal of FAD2, and deletion or substitutions in this protein region resulted in relaxed distribution and diffuse association with the ER. The distinct association of FAD2 with pre-cis-Golgi stacks in Arabidopsis root and leaf tissue is consistent with a contribution of FAD2 to membrane lipid homeostasis through the secretory pathway, as verified by an increased plasma membrane liquid phase order in the fad2 mutant.

Grützner, R.; König, K.; Horn, C.; Engler, C.; Laub, A.; Vogt, T.; Marillonnet, S.; A transient expression tool box for anthocyanin biosynthesis in Nicotiana benthamiana *Plant Biotechnol. J.* **22**, 1238-1250, (2024) DOI: 10.1111/pbi.14261

Abstract
Internet
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Transient expression in Nicotiana benthamiana offers a robust platform for the rapid production of complex secondary metabolites. It has proven highly effective in helping identify genes associated with pathways responsible for synthesizing various valuable natural compounds. While this approach has seen considerable success, it has yet to be applied to uncovering genes involved in anthocyanin biosynthetic pathways. This is because only a single anthocyanin, delphinidin 3-O-rutinoside, can be produced in N. benthamiana by activation of anthocyanin biosynthesis using transcription factors. The production of other anthocyanins would necessitate the suppression of certain endogenous flavonoid biosynthesis genes while transiently expressing others. In this work, we present a series of tools for the reconstitution of anthocyanin biosynthetic pathways in N. benthamiana leaves. These tools include constructs for the expression or silencing of anthocyanin biosynthetic genes and a mutant N. benthamiana line generated using CRISPR. By infiltration of defined sets of constructs, the basic anthocyanins pelargonidin 3-O-glucoside, cyanidin 3-O-glucoside and delphinidin 3-O-glucoside could be obtained in high amounts in a few days. Additionally, co-infiltration of supplementary pathway genes enabled the synthesis of more complex anthocyanins. These tools should be useful to identify genes involved in the biosynthesis of complex anthocyanins. They also make it possible to produce novel anthocyanins not found in nature. As an example, we reconstituted the pathway for biosynthesis of Arabidopsis anthocyanin A5, a cyanidin derivative and achieved the biosynthesis of the pelargonidin and delphinidin variants of A5, pelargonidin A5 and delphinidin A5.

Frey, M.; Bathe, U.; Meink, L.; Balcke, G. U.; Schmidt, J.; Frolov, A.; Soboleva, A.; Hassanin, A.; Davari, M. D.; Frank, O.; Schlagbauer, V.; Dawid, C.; Tissier, A.; Combinatorial biosynthesis in yeast leads to over 200 diterpenoids *Metab. Eng.* **82**, 193-200, (2024) DOI:

10.1016/j.ymben.2024.02.006

Abstract
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Diterpenoids form a diverse group of natural products, many of which are or could become pharmaceuticals or industrial chemicals. The modular character of diterpene biosynthesis and the promiscuity of the enzymes involved make combinatorial biosynthesis a promising approach to generate libraries of diverse diterpenoids. Here, we report on the combinatorial assembly in yeast of ten diterpene synthases producing (+)-copalyldiphosphate-derived backbones and four cytochrome P450 oxygenases (CYPs) in diverse combinations. This resulted in the production of over 200 diterpenoids. Based on literature and chemical database searches, 162 of these compounds can be considered new-to-Nature. The CYPs accepted most substrates they were given but remained regioselective with few exceptions. Our results provide the basis for the systematic exploration of the diterpenoid chemical space in yeast using sequence databases.

Ninck, S.; Halder, V.; Krahn, J. H.; Beisser, D.; Resch, S.; Dodds, I.; Scholtysik, R.; Bormann, J.; Sewald, L.; Gupta, M. D.; Heilmann, G.; Bhandari, D. D.; Morimoto, K.; Buscaill, P.; Hause, B.; van der Hoorn, R. A. L.; Kaschani, F.; Kaiser, M.; Chemoproteomics Reveals the Pan-HER Kinase Inhibitor Neratinib To Target an Arabidopsis Epoxide Hydrolase Related to Phytohormone Signaling *ACS Chem. Biol.* **18**, 1076-1088, (2023) DOI: 10.1021/acschembio.2c00322

Abstract
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Plant phytohormone pathways are regulated by an intricate network of signaling components and modulators, many of which still remain unknown. Here, we report a forward chemical genetics approach for the identification of functional SA agonists in Arabidopsis thaliana that revealed Neratinib (Ner), a covalent pan-HER kinase inhibitor drug in humans, as a modulator of SA signaling. Instead of a protein kinase, chemoproteomics unveiled that Ner covalently modifies a surface-exposed cysteine residue of Arabidopsis epoxide hydrolase isoform 7 (AtEH7), thereby triggering its allosteric inhibition. Physiologically, the Ner application induces jasmonate metabolism in an AtEH7-dependent manner as an early response. In addition, it modulates PATHOGENESIS RELATED 1 (PR1) expression as a hallmark of SA signaling activation as a later effect. AtEH7, however, is not the exclusive target for this physiological readout induced by Ner. Although the underlying molecular mechanisms of AtEH7-dependent modulation of jasmonate signaling and Ner-induced PR1-dependent activation of SA signaling and thus defense response regulation remain unknown, our present work illustrates the powerful combination of forward chemical genetics and chemical proteomics for identifying novel phytohormone signaling modulatory factors. It also suggests that marginally explored metabolic enzymes such as epoxide hydrolases may have further physiological roles in modulating signaling.

Zeng, M.; Krajinski, F.; Dam, N. M.; Hause, B.; Jarin-1, an inhibitor of JA-Ile biosynthesis in Arabidopsis thaliana , acts differently in other plant species *Plant Signaling & Behavior* **18**, e2273515, (2023) DOI: 10.1080/15592324.2023.2273515

Abstract
Internet
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Jasmonates (JAs), including jasmonic acid (JA) and its biologically active derivative JA-Ile, are lipid-derived plant signaling molecules. They govern plant responses to stresses, such as wounding and insect herbivory. Wounding elicits a rapid increase of JA and JA-Ile levels as well as the expression of JAR1, coding for the enzyme involved in JA-Ile biosynthesis. Endogenous increase and application of JAs, such as MeJA, a JA methylester, result in increased defense levels, often accompanied by diminished growth. A JA-Ile biosynthesis inhibitor, jarin-1, was shown to exclusively inhibit the JA-conjugating enzyme JAR1 in Arabidopsis thaliana. To investigate whether jarin-1 does function similarly in other plants, we tested this in Medicago truncatula, Solanum lycopersicum, and Brassica nigra seedlings in a root growth inhibition assay. Application of jarin-1 alleviated the inhibition of root growth after MeJA application in M. truncatula seedlings, proving that jarin-1 is biologically active in M. truncatula. Jarin-1 did not show, however, a similar effect in S. lycopersicum and B. nigra seedlings treated with MeJA. Even JA-Ile levels were not affected by application of jarin-1 in wounded leaf disks from S. lycopersicum. Based on these results, we conclude that the effect of jarin-1 is highly species-specific. Researchers intending to use jarin-1 for studying the function of JAR1 or JA-Ile in their model plants, must test its functionality before use.

Zeng, M.; Dam, N. M.; Hause, B.; MtEIN2 affects nitrate uptake and accumulation of photosynthetic pigments under phosphate and nitrate deficiency in Medicago truncatula *Physiol. Plant.* **175**, e13899, (2023) DOI: 10.1111/ppl.13899

Abstract
Internet
RIS
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Ethylene (ET) controls many facets of plant growth and development under abiotic and biotic stresses. MtEIN2, as a critical element of the ET signaling pathway, is essential in biotic interactions. However, the role of MtEIN2 in responding to abiotic stress, such as combined nutrient deficiency, is less known. To assess the role of ethylene signaling in nutrient uptake, we manipulated nitrate (NO3−) and phosphate (Pi) availability for wild-type (WT) and the ethylene-insensitive (MtEIN2-defective) mutant, sickle, in Medicago truncatula. We measured leaf biomass and photosynthetic pigments in WT and sickle to identify conditions leading to different responses in both genotypes. Under combined NO3− and Pi deficiency, sickle plants had higher chlorophyll and carotenoid contents than WT plants. Under these conditions, nitrate content and gene expression levels of nitrate transporters were higher in the sickle mutant than in the WT. This led to the conclusion that MtEIN2 is associated with nitrate uptake and the content of photosynthetic pigments under combined Pi and NO3−deficiency in M. truncatula. We conclude that ethylene perception plays a critical role in regulating the nutrient status of plants.

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