On the Inside

Lignin Bioengineering in Poplar

Lignified cell walls constitute an important renewable and sustainable feedstock for the production of fermentable sugars, biochemicals, and biomaterials. In biorefineries, plant cell wall polysaccharides are depolymerized into simple monomeric sugars, a process called saccharification. These sugars are subsequently fermented to ethanol or other compounds by microbes. However, the complex chemical composition and physical structure of plant cell walls hampers the efficient hydrolysis of lignocellulose. One of the major factors contributing to biomass recalcitrance of this sort is the presence of lignin, an aromatic polymer that provides strength and hydrophobicity to the cell wall. Lignin hinders the efficient enzymatic depolymerization of cellulose and hemicellulose into fermentable sugars by immobilizing the hydrolytic enzymes and physically limiting the access to their polysaccharide substrates. Although a number of pretreatments have been developed to remove lignin and consequently lower biomass recalcitrance, the pretreatment is still a relatively expensive step in the conversion process. In this regard, lignin bioengineering (e.g. engineering plants that either accumulate less lignin or produce lignin polymers more amenable to chemical degradation) holds promise to tailor plants with reduced biomass recalcitrance. Caffeoyl shikimate esterase (CSE) plays an essential role in lignin biosynthesis in Arabidopsis (*Arabidopsis thaliana*) and *Medicago truncatula*. **Van Acker et al. (pp. 1018–1039)** now report that the down-regulation of CSE in hybrid poplar (*Populus tremula* × *Populus alba*) resulted in up to 25% reduced lignin deposition, increased levels of p-hydroxyphenyl units in the lignin polymer, and a relatively higher cellulose content. The reduced lignin amount combined with the relative increase in cellulose content in the CSE down-regulated lines resulted in up to 62% more Glc released per plant upon limited saccharification when no pretreatment was applied and by up to 86% and 91% when acid and alkaline pretreatments were used. These results show that CSE is not only important for the lignification process in poplar but is also a promising target for the development of improved lignocellulosic biomass crops for biorefineries

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New Insights into Wound-Induced Callus Formation

Plants repair wound sites through the formation of unorganized cell masses called calli, which can also serve as progenitors of new organs. Callus formation and organ regeneration often entail cell-cycle re-entry of quiescent cells, which is achieved through the reactivation of core cell-cycle regulators CYCLIN (CYC) and CYCLIN-DEPENDENT KINASES (CDK). Recent studies have revealed how cells transduce wound signals to activate cell proliferation and callus formation. A set of AP2/ERF transcription factors called WOUND INDUCED DEDIFFERENTIATION1 (WIND1), WIND2, WIND3, and WIND4 plays key roles in wound-induced callus formation. **Ikeuchi et al. (pp. 1158–1174)** have combined transcriptome analysis with quantitative hormonal analysis to investigate how wounding induces callus formation in Arabidopsis. A time-course analysis revealed that wounding induces dynamic transcriptional changes, starting from rapid stress responses followed by the activation of metabolic processes and protein synthesis and subsequent activation of cell-cycle regulators. Gene ontology analyses suggest that wounding modifies the expression of hormone biosynthesis and response genes. A quantitative analysis of endogenous plant hormones revealed the accumulation of cytokinin prior to callus formation. Mutants defective in cytokinin synthesis or signaling display reduced efficiency in callus formation, indicating that de novo synthesis of cytokinin is critical for wound-induced callus formation. Other evidence presented suggests a possible role for ETHYLENE RESPONSE FACTOR 115 (ERF115) in callus formation. The authors demonstrate that type-B ARABIDOPSIS RESPONSE REGULATOR (ARR)-mediated cytokinin signaling regulates the expression of *CYCLIN D3;1* (*CYCD3;1*) and that mutations in *CYCD3;1* and its homologs *CYCD3;2-3* cause defects in callus formation. In toto, the results presented provide novel mechanistic insights into how wounding reactivates cell proliferation during callus formation.

Chemical Defenses of Maize Roots

Of the many classes of natural products produced by plants, terpenoids are the most structurally diverse, with well over 25,000 established compounds. In maize (*Zea mays*), terpene olefins are nearly ubiquitous components of induced volatile emissions following biotic stress. In contrast to our understanding of foliar volatiles, much less is known about the volatile emissions of roots. To better understand below-ground defenses in the field, **Ding et al. (pp. 1455–1468)** performed root metabolomic profiling and uncovered unexpectedly high levels of the sesquiterpene volatile β-selinene and the corresponding nonvolatile antibiotic derivative β-costic acid. The authors identify *terpene synthase21* (*ZmTps21*) as a β-costic acid pathway candidate gene. For biochemical validation, a full-length *ZmTps21* was cloned, heterologously expressed in *Escherichia coli*, and demonstrated to cyclize farnesyl diphosphate, yielding β-selinene as the dominant product. Numerous β-costic acid-deficient inbred lines were found to harbor *Zmtps21* pseudogenes lacking conserved motifs required for farnesyl diphosphate cyclase activity. Consistent with a role in defending against plant pathogens, *ZmTps21* transcripts accumulate strongly following fungal elicitation. Roots containing functional *ZmTps21* alleles when challenged in the field displayed β-costic acid levels exceeding those required to inhibit the growth of five different fungal pathogens and rootworm larvae (*Diabrotica balteata*).

Xylem Sap Surface Tension and Hydraulic Safety

Xylem embolisms are induced by drought stress and/or freezing stress by means of "air-seeding," that is, the aspiration of gaseous bubbles into xylem conduits from adjacent gas-filled compartments via pits. At water potentials less negative than the threshold for air seeding, the air-water interface is stabilized by the surface tension (γ) of the xylem sap. But is the γ of the xylem

sap constant from season to season? **Losso et al. (pp. 1135–1143)** studied seasonal changes in xylem sap γ in *Picea abies* and *Pinus mugo* growing at the alpine timberline. They analyzed the vulnerability of these trees to drought-induced embolism using solutions of different γs. and estimated the potential effect of seasonal changes in γ on hydraulic vulnerability. In both species, xylem sap γ showed pronounced seasonal changes. Variations in γ within the observed physiological range also causes changes in hydraulic vulnerability. Solutions with low γ caused higher vulnerability to drought-induced xylem embolism. The authors also noted pronounced effects of changes in xylem sap γ on the hydraulic safety of trees "in situ." Further studies are necessary to understand the variability of xylem sap γ and its effects on plant hydraulics.

A MicroRNA Affecting Grain Yield in Rice

MicroRNAs (miRNAs), a class of abundant small noncoding RNAs, have been identified as important regulators of gene expression in plants, affecting many aspects of plant development. Recently, several miRNAs have been reported to regulate rice grain yield. A previous study revealed that miR397 regulates rice grain yield by affecting a blue copper protein, laccase. This mechanism is conserved between monocots and dicots, suggesting that miRNA mediation of blue copper protein could be a novel method for improving rice yield. A genome wide screening of miRNA expression during embryogenesis and postembryogenesis found that among all the known miRNAs, only one conserved miRNA, miR408, showed the same expression pattern as that of miR397 during embryogenesis. Coincidently, miR408 also targets the blue copper protein. Thus, **Zhang et al. (pp. 1175–1185)** examined the question of whether miR408 could regulate rice grain yield too? MiR408 has been reported to target a variety of blue copper protein members, including those in the phytocyanin family. Both phytocyanin and laccase are blue copper proteins, albeit of different types. The authors report that the elevated expression of OsmiR408 positively regulates grain yield in rice by increasing panicle branches and grain number. They further showed that OsmiR408 regulates grain yield by down-regulating its downstream target, *OsUCL8*, which is a gene of the phytocyanin family. The knockdown or knockout mutants of *OsUCL8* demonstrated increased grain yield, while the overexpression of *OsUCL8* results in less. Further studies revealed that the cleavage of OsUCL8 by miR408 affects copper homeostasis in the plant cell, which, in turn, affects the abundance of plastocyanin proteins and photosynthesis in rice.

Nitric Oxide and Diatoms

All gases in the N cycle, including nitric oxide (NO), are present in oceans, either because of gas exchanges at the air-water interface or because they are produced within oceans themselves. NO, a physiologically important gaseous transmitter, is generated in seawater by nonbiological photochemical reactions, large-scale electrical discharges, and enzymatic activities in organisms living in the aerobic photic zone or in oxygen minimum zones. The impact of marine NO is of special interest in the case of diatoms because it has previously been suggested to act in population size control. More specifically, NO was reported to mediate programmed cell death in response to high concentrations of the diatom-derived aldehyde 2E,4E/Z-decadienal (DD). **Dolch et al. (pp. 1407–1423)** have re-examined these claims using a strain of the marine diatom *Phaeodactylum*. Two major enzymatic pathways can produce NO in aerobic conditions: these involve either a nitric oxide synthase (NOS) that uses Arg as a substrate or a nitrate reductase (NR) that uses nitrite as a substrate. The authors could not confirm previous reports of the production of NO by a nitric oxide synthase (NOS)-like activity in *Phaeodactylum* (indeed, the gene for such an enzyme is lacking from the genome). Rather, the authors found that NO is produced in *Phaeodactylum* via a nitrite-dependent pathway. The authors caution, however, that not all diatoms lack an *NOS* gene and that the nitrite-sensing system might be different in *NOS*-containing diatoms. NO does, however, redirect carbon flux toward the production of triacylglycerol, at least partly via transcriptomic reprogramming. This NO-dependent remodeling of carbon metabolism seems to depend, at least partly, on the presence of the Orn-urea pathway, producing fumarate as a side product. The authors propose a revision of the physiological and ecophysiological role of NO in diatoms and suggest that its role is related to the environmental nitrogen status and more specifically the level of nitrite. They do not rule the possibility that an anthropogenic increase of NO in the environment could alter N assimilation systems and act as an important stressor at the ecosystem level.

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