On the Inside

Trichomes: The Source of Heavy Metal in Tobacco Smoke?

Smoking of tobacco (*Nicotiana tabacum*) leaves is one of the principal routes of exposure to heavy metals (Fig. 1). Little is known about the mechanisms of heavy-metal accumulation and detoxification in tobacco. Recently, it was shown that the trichomes of tobacco exposed to Cd^{2+} and Ca^{2+} produced calcium (Ca)/cadmium (Cd)-containing grains. Other effects of Cd exposure were a retardation of plant growth and a 2-fold increase of the number of trichomes in comparison with untreated plants. An increased concentration of Ca in the nutrient medium was found to have a protective effect toward Cd toxicity and enhanced the production of the grains. The Ca/Cd-containing grains were 20 to 150 μm in diameter, and formed on the head cells of both the short and long trichomes of tobacco leaves. Thus, these studies revealed a new function of tobacco trichomes, the excretion of Cd in the form of particles. **Sarret et al. (pp. 1021–1034)** bring to bear an arsenal of cutting-edge techniques to examine the question of whether the trichomes of tobacco leaves also play a role in the responses of tobacco plants to toxic levels of zinc (Zn). Zn exposure resulted in toxicity signs in plants, and these damages were partly reduced by a Ca supplement. Confocal imaging of intracellular Zn using a fluorescent indicator showed that Zn was preferentially accumulated in trichomes. Exposure to Zn alone and Zn plus Ca increased the trichome density and induced the production of Ca/Zn mineral grains on the head cells of trichomes. These grains were aggregates of submicrometer-sized crystals and poorly crystalline material, and contained Ca as major element. Micro x-ray diffraction revealed that the large majority of the grains were composed essentially of metal-substituted calcite (calcium carbonate). Thus, the production of Zn-containing biogenic calcite and other Zn compounds through the trichomes is a novel mechanism involved in Zn detoxification. This study also illustrates the potential of laterally resolved x-ray synchrotron radiation techniques to study biomineralization and metal homeostasis processes in plants.

Figure 1. Heavy metal-excreting trichomes appear to be a major source of heavy-metal exposure from tobacco smoke. The painting is *Woman with a Cigarette* by Pablo Picasso.

Membrane Lipid Saturation and Chilling Sensitivity: Surprising Results

Some Arabidopsis (*Arabidopsis thaliana*) mutants that exhibit decreased thylakoid unsaturation are substantially indistinguishable from wild type when grown at 22°C, but exhibit defects in biogenesis and maintenance of chloroplasts at temperatures below 5°C. A role for thylakoid unsaturation in maintaining photosynthetic function at low temperatures is also suggested by experiments in which transgenic expression of fatty acid desaturases in chilling-sensitive plant species resulted in increased survival of plants at low temperatures. The Arabidopsis mutant *fatty acid biosynthesis 1* (*fab1*) that is partially deficient in β-ketoacyl-synthase II (KAS2) activity exhibits a distinct low-temperature phenotype. This mutant contains increased levels of 16:0 due to a mutation in the *KAS2* gene, which encodes the condensing enzyme that catalyzes the first step in elongation of 16:0 to 18:0 during fatty acid synthesis. In *fab1* mutants, the 16:0 fraction of the thylakoid phospholipid phosphatidylglycerol (PG) is increased from 20% in wild type to 41% in *fab1*. This is significant because increased 16:0 in PG, and more specifically the sum of 16:0 + 18:0 + 16:1, Δ3 trans (sometimes referred to as high-melting-point fatty acids), has been correlated with chilling sensitivity through surveys of chilling-tolerant and chilling-sensitive plant species. Typically, plants containing more than 60% high-melting-point fatty acids in PG have been shown to be chilling sensitive. The *fab1* mutant contains 69% high-melting-point fatty acids in PG—a higher percentage than is found in many chilling-sensitive plants. However, *fab1* plants were completely unaffected, when compared with wild-type controls, by a range of chilling treatments that quickly killed other chilling-sensitive plants. Instead, *fab1* plants are damaged only by long-term (>10 day) exposure to low temperature. Are the elevated levels of high-melting-point fatty acids in PG the direct cause of the damage and death of *fab1* plants at 2°C? Surprisingly, the answer is no. **Barkan et al. (pp. 1012–1020)** have isolated suppressor mutations that rescue *fab1* from death at low temperatures. One of the suppressors is an allele of *fad5*, a mutant that has decreased chloroplast 16:0 Δ7-desaturase and, hence, more saturated chloroplast membrane lipids. The overall leaf fatty acid composition of the rescued line contained 31% 16:0 compared with 23% in *fab1* and 17% in wild type. Based on the biophysical characteristics of saturated and unsaturated fatty acids, the increased 16:0 in *fab1 fad5-2* plants would be expected to exacerbate, rather than ameliorate, low-temperature damage. The authors speculate that changes in the shape of other lipids may compensate for disruptive changes in the shape of PG molecules induced by the *fab1* mutation, for example, by altering the packing relationships between the thylakoid lipids and membrane proteins of the photosynthetic complexes. Clearly, more needs to be learned about the relationship between thylakoid membrane saturation and chilling sensitivity.

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Identification of a Phosphate-Overaccumulating Mutation

The Arabidopsis mutant *pho2*, which is defective in inorganic phosphate (Pi) homeostasis, was identified by screening the Pi content of shoots. Even though normal Pi concentrations are maintained in the roots of *pho2* mutants, the shoots accumulate excessive amounts of Pi and exhibited Pi-toxic symptoms. Recently, it was reported that a microRNA, miR399, controls Pi homeostasis by regulating the expression of a ubiquitin-conjugating E2 enzyme (UBC24) in Arabidopsis. The accumulation of *UBC24* mRNA was suppressed by the targeting of miR399, whose expression is up-regulated by Pi starvation. Intriguingly, several characteristics of Pi toxicity in the *pho2* mutant are similar to those in miR399-overexpressing and *UBC24* T-DNA knockout plants: both Pi uptake and translocation of Pi from roots to shoots is increased and Pi remobilization within leaves is impaired. In this issue, both **Aung et al. (pp. 1000–1011)** and **Bari et al. (pp. 988–999)** demonstrate that the Pi overaccumulator *pho2* is caused by a single nucleotide mutation resulting in early termination within the *UBC24* gene. No UBC24 protein was detected in the *pho2* mutant, and the phenotype of the *pho2* mutation could be rescued by introduction of a wild-type copy of *UBC24*. The combined results of these two research groups provide many further insights into the workings of this peculiar mutant. It was demonstrated by micrografting experiments that a *pho2* root genotype is sufficient to yield leaf Pi accumulation, suggesting that Pi toxicity in this mutant arises from increased Pi uptake and translocation of Pi from roots to shoots. Furthermore, miR399 and UBC24 were colocalized in the vascular cylinder. Bari et al. present a working model for the mechanism of Pi sensing in higher plants in which PHR1, a MYB factor that has previously been shown to be required for induction of a small number of genes under Pi starvation, takes a central role and the downstream miR399/PHO2 pathway regulates the expression of only a subset of the phosphate starvation-induced genes. The identification of putative *PHO2* orthologs containing five miR399 binding sites in other higher plants and the demonstration of Pi-dependent miR399 expression in rice (*Oryza sativa*) suggest that this Pi starvation-signaling pathway may be highly conserved throughout the plant kingdom.

Brassinosteroid Insensitivity Increases Rice Production

The major factor underlying the success of the Green Revolution was the introduction of high-yielding semidwarf cultivars of wheat (*Triticum aestivum*) and rice. The dwarf phenotypes of the Green Revolution cultivars were largely traceable to disruptions in GA signaling or biosynthesis. Recently, however, another important target for producing high-yielding semidwarf cultivars was identified in barley (*Hordeum vulgare*). The semidwarf *uzu* phenotype of barley is brassinosteroid (BR) insensitive and is caused by a missense mutation in *HvBRI1*, an ortholog of the *Arabidopsis* gene *BRASSINOSTEROID INSENSITIVE1* (*BRI1*). In contrast to barley, the loss-of-function mutants of a rice *BRI1* ortholog (*OsBRI1*), namely, *d61*, show a range of phenotypes. Although the weak alleles *d61-1* and *d61-2* exhibit agronomically useful traits, such as semidwarf stature, erect leaves, and elongated neck internodes, they also exhibit, unfortunately, morphological alterations in their reproductive organs and reduced grain yield. Of nine *d61* alleles identified by **Morinaka et al. (pp. 924–931)**, the weakest, *d61-7*, confers agronomically important traits, such as semidwarf stature and erect leaves. The biomass produced by wild type was 38% higher than that of *d61-7* at harvest under conventional planting density, whereas, as the situation was reversed at high planting densities, the biomass of *d61-7* was 35% higher than that of wild type. However, the small grain size of *d61-7* countered any increase in grain yield, leading to the same grain yield as that of wild type even at high density. The authors therefore produced transgenic rice with partial suppression of endogenous *OsBRI1* expression. Several of these transformants, although of the same height as wild type, exhibited the desirable phenotype of erect leaves. The estimated grain yield of these transformants was about 30% higher than that of wild type at high growth densities. These results demonstrate the feasibility of generating erect-leaved plants by modifying the expression of the BR receptor gene in transgenic rice plants.

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CORRECTIONS

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Abdulrazzak N., Pollet B., Ehlting J., Larsen K., Asnaghi C., Ronseau S., Proux C., Erhardt M., Seltzer V., Renou J.-P., Ullman P., Pauly M., Lapierre C., and Werck-Reichhart D. A *coumaroyl-ester-3-hydroxylase* Insertion Mutant Reveals the Existence of Nonredundant *meta*-Hydroxylation Pathways and Essential Roles for Phenolic Precursors in Cell Expansion and Plant Growth.

The authors regret that this article contains a description of immunofluorescence/confocal microscopy methodology that was not actually used for this work. In addition, this methodology was given without proper credit to Sugimoto et al. (K. Sugimoto, R.E. Williamson, G.O. Wasteneys [2000] Plant Physiol **124:** 1493–1506), who developed the protocol. The correct immunofluorescence/confocal microscopy methodology used for this article is described below. The authors apologize for this error and any inconvenience it may have caused.

Immunofluorescence Visualization of the Microtubules

Roots of 2-week-old seedlings were fixed in 2% (v/v) paraformaldehyde and 0.5% (v/v) glutaraldehyde in PEMT buffer (100 mM PIPES, 4 mM EGTA, 4 mM $MgSO_4$, 0.05% [v/v] Triton X-100, pH 7.2) for 40 min, and rinsed in PEMT buffer three times for 10 min. Roots were postfixed in cold methanol (−20°C) for 10 min on ice, rehydrated for 10 min in 1× PBS (136 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4), and treated for 20 min with $NaBH_4$ (1 mg/mL) diluted in 1× PBS. Fixed roots were digested for 10 min with 0.2% (w/v) pectolyase, 1% (w/v) macerozyme, 3% (w/v) caylase diluted 10 times in digestion buffer (25 mM MES, 8 mM $CaCl_2$, 600 mM mannitol, pH 5.5). After three washes in PBSG buffer (1× PBS, 50 mM Gly), roots were incubated for 20 min in 5% normal goat serum diluted in PBSG to saturate nonspecific sites, then incubated with primary antibodies directed against α-tubulin (Molecular Probes) in PBSG at 4°C overnight and washed three times for 5 min in PBSG buffer. Samples were incubated for 1 h at room temperature with secondary antibodies coupled to Alexa Fluor 488 (Molecular Probes) and washed three times in PBSG buffer. Roots were mounted in Mowiol containing DABCO (100 mg/mL).

Observations were done using a Zeiss LSM510 confocal laser scanning microscope equipped with argon and helium/neon lasers and with a C-APOCHROMAT (× 63, 1.2 numerical aperture water immersion lens). Excitation/emission wavelengths were 488/bandpass 505 to 550 nm for Alexa 488. Image processing was done using LSM510 version 2.8 (Zeiss), ImageJ (W.S. Rasband; National Institutes of Health), and Photoshop 6.0 (Adobe Systems).

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Plant Physiology regrets that the credit line was not included with the image of Pablo Picasso's *Woman with a Cigarette* in July's On the Inside feature. The Estate of Pablo Picasso has graciously granted permission to ASPB for use of this image in the print and online journal. The credit line for this image is as follows: © 2006 Estate of Pablo Picasso/Artists Rights Society (ARS), New York.