On the Inside

Lubrication Affects Petal Morphogenesis

A peek inside an unopened floral bud reveals one of nature's marvels: How do all these floral parts form so perfectly in such a crowded space? In Arabidopsis (*Arabidopsis thaliana*), petal primordia arise concurrently with stamen primordia. Stamens grow faster than petals until anthers fill the upper internal space created by the protective dome formed by the closed sepals. Soon thereafter, petal growth is accelerated, and petals elongate through a narrow space generated by the sepals and anthers. **Takeda et al. (pp. 1242–1250)** have discovered a mutant, *folded petals1* (*fop1*), whose petals are folded at the mature stage, but whose overall size and shape are normal. During elongation, the *fop1* petals fuse to the sepal surface at several sites. The normally conical-shaped petal epidermal cells are flattened in the *fop1* mutant, as if the apices of the cones of each cell have been pressed in. The authors found that the petals become stuck in the narrow space between the sepals and stamens in the bud, causing the petal to fold at flower opening. Surgical or genetic removal of sepals in young buds restores the regular growth of petals, suggesting that the narrow space between sepals and stamens is critical for petal folding in the *fop1* mutant. *FOP1* encodes a member of a wax ester synthase/diacylglycerol acyltransferase gene family, which is expressed in elongating petals and localized to the plasma membrane. The authors suggest that the *FOP1* products synthesized in the petal epidermis may act as a lubricant, enabling uninhibited growth of the petals as they extend between the sepals and the anthers.

Trehalase Overexpression Enhances Drought Tolerance

Trehalose is a nonreducing sugar that has been implicated in osmoregulation and stress protection in many bacteria and fungi. Among the vascular plants, only a few desiccation-tolerant resurrection plants accumulate substantial amounts of trehalose. In most other higher plants, only trace amounts of trehalose are detected, suggesting that there is insufficient trehalose for it to act as a compatible solute during environmental stress. Many attempts, therefore, have been made to enhance the stress tolerance of model plants and crops by introducing trehalose biosynthesis genes of yeast (*Saccharomyces cerevisiae*) or bacterial origin. In general, the stress tolerance of these plants increased, although trehalose levels remained low. In most cases, the plants exhibited aberrant phenotypes such as stunted roots, lancet-shaped leaves, and growth retardation. The undesired abnormalities in these plants were ascribed to the perturbation of developmental processes by altered levels of the important signal metabolite trehalose-6-P. In attempting a novel approach toward engineering drought tolerance in Arabidopsis plants by manipulating the expression and activity of the endogenous trehalase AtTRE1, **Van Houtte et al. (pp. 1158–1171)** have uncovered some more hurdles in the dream of enhancing plant drought tolerance by elevating endogenous levels of trehalose. The authors constructed *AtTRE1* overexpressing and *Attre1* mutant lines and tested their performances in drought stress assays. As expected, *AtTRE1* overexpressing and *Attre1* mutants had decreased and increased trehalose levels, respectively. Surprisingly, however, *AtTRE1* overexpressing lines recovered better after drought stress, whereas *Attre1* mutants exhibited a drought-susceptible phenotype. The leaves of *AtTRE1* overexpressing plants were found to have a better water-retaining capacity and a greater sensitivity to abscisic acid. The opposite was found to be the case for *Attre1* mutants. These results show that the overexpression of plant trehalase improves drought stress tolerance in Arabidopsis and that trehalase plays a role in the regulation of stomatal closure during plant drought stress responses.

Shoot Regeneration by Phenyl-Adenine

The capacity to regenerate shoots is a critical step in many tissue culture and biotechnological protocols. Facile shoot regeneration in vitro is a fairly common trait, although some species remain recalcitrant. Cytokinins are the plant growth regulators most typically associated with in vitro shoot regeneration. **Motte et al. (pp. 1229–1241)** therefore set out to search for novel shoot-inducing compounds by combining a chemical screen with a previously described regeneration procedure. To identify compounds that promote shoot regeneration, they screened a library of 10,000 small molecules. The bioassay consisted of a two-step regeneration protocol that was adjusted and optimized for high-throughput manipulations of root explants of Arabidopsis carrying a shoot regeneration marker gene. The screen revealed a single compound, the cytokinin-like phenyl-adenine (Phe-Ade), as a potent inducer of adventitious shoots. Although Phe-Ade triggered diverse cytokinin-dependent phenotypical responses, it had the desirable traits of not inhibiting shoot growth or being cytotoxic at high concentrations. Transcript profiling of cytokinin-related genes revealed that Phe-Ade treatment established a typical cytokinin response. Moreover, Phe-Ade activated the cytokinin receptors ARABIDOPSIS HISTIDINE KINASE3 (AHK3) and AHK4 in a bacterial receptor assay, albeit at relatively high concentrations, illustrating that it exerts genuine but weak cytokinin activity. Finally, the authors demonstrated that Phe-Ade acts a strong competitive inhibitor of cytokinin-degrading enzymes, thereby leading to an accumulation of endogenous cytokinins. In effect, Phe-Ade is a weak cytokinin that strongly inhibits endogenous cytokinin degradation. Given its properties, Phe-Ade would appear to be a most promising compound to explore in tissue culture protocols.

Nitrogen Fixation in Unicellular Cyanobacteria

Nitrogen fixation is largely achieved by biological means through the activity of microorganisms. Nitrogenase, the enzyme complex involved in biological nitrogen fixation reaction, is extremely

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sensitive to oxygen. Thus, most nitrogen-fixing microbes engage in this process only when suitably anaerobic conditions are achieved. For example, some filamentous cyanobacteria develop specialized cells called heterocysts that allow the spatial segregation of photosynthesis and nitrogen fixation. Heterocysts have higher rates of respiratory oxygen consumption, which results in a virtually anoxic environment conducive for the nitrogenase enzyme. However, some nitrogen-fixing (diazotrophic) microbes have the advantage of being able to fix nitrogen even in aerobic environments. Outstanding among these are certain cyanobacteria such as the unicellular cyanobacterial genus *Cyanothece* that thrive in marine as well as terrestrial environments. Another unusual attribute of *Cyanothece* spp. is their ability to produce molecular hydrogen at exceptionally high rates under aerobic conditions. **Bandyopadhyay et al. (pp. 1334–1346)** focus their attention on the patterns of nitrogen fixation and respiration in six different *Cyanothece* spp. strains, in an effort to elucidate the underlying differences and similarities in strains with similar genotypic but varied ecological backgrounds. Their study reveals that unicellular diazotrophic cyanobacteria with the same genotypic background exhibit considerable diversity in the diurnal patterns of central metabolic processes. The variations appear to be controlled by intracellular metabolic signals specific to each strain, for example the intracellular concentration of carbon, nitrogen, or oxygen, which in turn control the ability of the cells to fix nitrogen. High rates of respiration, adequate supply of energy and reductants from efficient photosynthesis, and unidentified components of the nitrogen-fixing machinery also appear to be factors contributing to the unique aerobic hydrogen producing ability of the genus *Cyanothece*.

Increasing Seed Longevity and Vigor

The generation and accumulation of spontaneously damaged proteins in seeds due to aging or stresses often adversely affects their vigor and viability. Such damaged proteins are thought to arise primarily because of spontaneous covalent modifications of existing proteins. Among such covalent protein modifications, conversion of L-aspartyl or asparaginyl residues to abnormal isoaspartyl residues in proteins is quite prevalent. PROTEIN L-ISOASPARTYL *O*-METHYLTRANSFERASE (PIMT) is an enzyme that converts abnormal L-isoaspartyl residues to normal aspartyl residues, thereby serving to repair damaged proteins. PIMT is an ancient and highly conserved enzyme that is widely distributed in phylogenetically divergent organisms including eubacteria, archaebacteria, protozoa, fungi, nematodes, mammals, and plants. PIMT in plants is unusual because it is encoded by two divergent genes (*PIMT1* and *PIMT2*). **Verma et al. (pp. 1141–1157)** have isolated the *PIMT2* gene (*CaPIMT2*) from chickpea (*Cicer arietinum*), a species that exhibits significant increases in isoaspartyl residues in seed proteins coupled with reduced germination vigor under artificial aging conditions. Using a variety of techniques, the authors demonstrate that both CaPIMT2 isoforms localize predominantly in the nucleus, whereas CaPIMT1 localizes to the cytosol. CaPIMT2 enhances seed vigor and longevity by repairing abnormal isoaspartyl residues predominantly in nuclear proteins in Arabidopsis, whereas CaPIMT1 enhances seed vigor and longevity by repairing such abnormal proteins mainly in the cytosolic fraction. These data suggest that *CaPIMT2* has most likely evolved through gene duplication, followed by subfunctionalization to specialize in repairing the nuclear proteome. Expression analysis revealed that *CaPIMT2* is differentially regulated by stresses and abscisic acid.

Callose and Mildew Resistance

A common response by plants to fungal attack is deposition of callose, a (1,3)-β-glucan polymer, in the form of cell wall thickenings called papillae, at the site of wall penetration. Although papillar callose deposition is induced in essentially all plants following pathogen challenge, the importance of callose deposits in pathogen resistance is still debated. Because callose-rich papillae have also been found at sites where a pathogen has successfully penetrated, callose deposits apparently cannot always prevent or sufficiently slow pathogen ingress. Moreover, Arabidopsis mutants lacking a stress-induced type of callose synthase (*POWDERY MILDEW RESISTANT4* [*PMR4*]) did not deposit callose at sites of attempted penetration, but unexpectedly demonstrated an increased resistance to powdery mildew (*Blumeria graminis*). Double mutant and microarray analyses showed that hyperactivation of the salicylic acid pathway caused the high resistance of the *pmr4* mutant. **Ellinger et al. (pp. 1433–1444)** present evidence that it is not so much the final amount of callose deposited that is critical in deterring pathogen invasion but rather the early onset of callose deposition. They generated transgenic Arabidopsis lines that constitutively express *PMR4*. In these lines, they detected callose synthase activity that was 4 times higher than that in wild-type plants 6 h after inoculation with the virulent powdery mildew *Golovinomyces cichoracearum*. The callose synthase activity was correlated with enlarged callose deposits. Because complete penetration resistance was achieved, neither the salicylic acid-dependent nor the jasmonate-dependent pathways were induced. Thus, callose synthesis and deposition can play an active role in plant resistance to powdery mildews.

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