On the Inside

Increasing the Shelf Life of Cassava Roots

Cassava (*Manihot esculenta*) is a woody shrub of the Euphorbiaceae family, grown mainly for its edible tuberous roots. In the tropics, where it is a major staple food crop, cassava is the fourth most important source of calories. In addition to its use as a staple food crop, cassava is also a potential biofuel crop because of its high starch production. One of the major constraints facing the large-scale production of cassava roots is the rapid postharvest physiological deterioration (PPD) that occurs within 72 h after harvest. PPD is typically initiated by mechanical damage, which occurs during harvesting. One of the earliest recognized biochemical events during the initiation of PPD is a rapid burst of reactive oxygen species (ROS) accumulation. Although the role of oxidative stress in PPD has been established, the early events that trigger the oxidative burst have not been identified. **Zidenga et al. (pp. 1396–1407)** have examined the role of cyanogens in the oxidative burst observed during the onset of PPD. They show that cyanide released during mechanical damage in cassava roots results in the buildup of ROS. Furthermore, they show that the overexpression of alternative oxidase (a cyanide-resistant terminal oxidase in plants) in cassava storage roots reduces accumulation of ROS and delays PPD by 10 to 21 d under greenhouse and field conditions.

Climacteric versus Nonclimacteric Ripening: A Transcriptomic Analysis

Fruits are classified into two physiological groups, "climacteric" or "nonclimacteric," according to their respiration patterns and reliance on ethylene biosynthesis during ripening. Climacteric fruits show an increase in respiration rate and ethylene formation during ripening. Nonclimacteric fruits exhibit neither the respiratory burst nor an elevated ethylene synthesis during ripening. Although some peppers are climacteric, Habanero pepper (*Capsicum chilense* 'Habanero') is not. To unravel the similarities and differences of the underlying regulatory processes involved in climacteric versus nonclimacteric fruit ripening, **Osorio et al. (pp. 1713–1729)** have performed comparative analyses of transcript and metabolite levels from two solanaceous crops, climacteric tomato (*Solanum lycopersicum*) fruits and nonclimacteric Habanero peppers. To this end, they conducted a combined gas chromatography-mass spectrometry and heterologous microarray hybridization assay in tomato and Habanero peppers across various stages of development and ripening. The major finding of the study is that genes involved in ethylene biosynthesis were not induced in Habanero pepper fruits during ripening. Nevertheless, genes downstream of ethylene perception such as those involved in cell wall metabolism and carotenoid biosynthesis, and the *never ripe receptor* (an ethylene receptor) gene were clearly induced in Habanero pepper as in tomato fruit. Although signaling sensitivity or actual signals may differ between climacteric and nonclimacteric fruit, the evidence presented in this study suggests that the activation of a common set of ripening genes influences metabolic traits.

A Critical Regulator of Plant Immunity

Plant cells have tough cell walls that hinder invasion by pathogens. However, some pathogens can invade the plant by breaking these cell walls, activating a first line of immune defense. Since the receptors on the cell membrane that are involved in this first line of immune response can recognize many patterns of molecules, they are called pattern recognition receptors (PRR) receptors. Previously, a screen searching for mutants with constitutively activated defense responses that are independent of NPR1 (for Nonexpressor of Pathogenesis-Related Genes1), an essential regulator of plant systemic acquired resistance, led to the identification of *snc2-1D* (for *suppressor of npr1, constitutive 2-1D*), a gain-of-function mutant. This mutant exhibits dwarf morphology, accumulates high levels of salicylic acid and Pathogenesis-Related (PR) proteins, and displays enhanced resistance to pathogens such as *Pseudomonas syringae* pv *tomato* DC3000, the cause of bacterial speck on tomato and Arabidopsis (*Arabidopsis thaliana*). To identify defense-signaling components downstream of *SNC2*, **Yang et al. (pp. 1857–1865)** carried out a suppressor screen in the *snc2-1D* mutant background. Map-based cloning of one of the suppressor genes, *BDA1* (for *BIAN DA1*; "becoming big" in Chinese) revealed that it encodes a novel ankyrin-repeat transmembrane protein. Loss-of-function mutations in *BDA1* suppress the dwarf morphology and constitutive defense responses in *snc2-1D npr1-1* and result in enhanced susceptibility to bacterial pathogens. In contrast, a gain-of-function allele of *bda1* was found to constitutively activate cell death and defense responses. These findings suggest that *BDA1* is a critical signaling component that functions downstream of *SNC2* to regulate PAMP-triggered plant immunity.

Do Pin Proteins Determine Phyllotaxy?

Phyllotaxis, the regular arrangement of leaves and flowers around the stem, is a key feature of plant architecture. The divergence angles between successive organs are species dependent, but most frequently tend toward 137.5°, which results in spiral phyllotaxis. Currently, the leading model of phyllotaxis proposes that the spatiotemporal regulation of organ initiation is controlled by a positive feedback loop between the plant hormone auxin and its efflux carrier PIN-FORMED1 (PIN1). Organ initiation is severely impaired in the inflorescence meristem of *pin1* mutants, but *pin1* plants still produce both cotyledons and true leaves during vegetative growth. Single mutants of other PINS display no obviously altered shoot phenotype under normal growth conditions. To understand the regulatory mechanisms controlling leaf initiation in Arabidopsis rosettes, **Guenot et al. (pp. 1501–1510)** have characterized the vegetative *pin1* phenotype in detail. They show that although the timing of leaf initiation in vegetative *pin1* mutants is variable, and divergence angles clearly deviate from the canonical 137° value, leaves are not positioned at random during early de-

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velopmental stages. Their data further indicate that other PIN proteins, which might potentially substitute for PIN1 in the Arabidopsis rosette, are not likely to explain the observed residual leaf positioning mechanism. Thus, leaf phyllotaxis appears to be more complex than suggested by current mechanistic models.

A Step toward Promoting C_4-Type Leaf Anatomy in C_3 Oat

The adaptation of C_4 photosynthesis increases the efficiency of photosynthesis by reducing photorespiration, which, in C_3 individuals, can reduce photosynthetic efficiency by up to 40%. The increased productivity of C_4 plants has stimulated interest in placing the C_4 pathway into C_3 crops to raise yield potential. C_4 photosynthesis has evolved in at least 66 angiosperm lineages and involves alterations to the biochemistry, cell biology, and the development of leaves. The characteristic "Kranz" anatomy of most C_4 leaves was discovered in the 1890s, but the genetic basis of these traits remains poorly defined. Oat × maize addition (OMA) lines allow the effects of individual maize (*Zea mays*; C_4) chromosomes to be investigated in an oat (*Avena sativa*; C_3) genetic background, substantially reducing the complexity of the maize genome and permitting traits of interest to be mapped to individual chromosomes. Therefore, OMAs are an interesting system with which to investigate the extent to which leaf anatomy can be modified in C_3 species. **Tolley et al. (pp. 1418–1427)** have used OMA lines to study the impact of individual maize chromosomes on the control of leaf development in C_3 oat. Specifically, the authors have examined the effect of maize chromosomal introductions on leaf morphology, chloroplast development, and carbon assimilation in C_3 oat leaves. Although there is no indication of a simultaneous change to C_4 biochemistry, leaf anatomy, and ultrastructure in any of the OMA lines, the C_3 oat leaf is modified at multiple levels in some OMA lines. Maize genes encoding important C_4 enzymes, including phospho*enol*pyruvate carboxylase, pyruvate, orthophosphate dikinase, and the 2′-oxoglutarate/malate transporter, are expressed in oat and generate transcripts of the correct size. Moreover, three maize chromosomes independently cause increases in vein density, and maize chromosome 3 results in larger bundle sheath cells with increased cell wall lipid deposition in oat leaves. These data heighten the prospect that aspects of C_4 biology can be successfully integrated into leaves of C_3 crops.

Reducing Transgene Escape in Populus spp.

A major concern surrounding field trials and the commercial deployment of transgenic trees is the potential for transgene introgression into the gene pool of wild relatives via cross-pollination. Eliminating viable pollen production would be an effective containment method to reduce direct gene flow from transgenic trees to their wild relatives. To date, only limited success has been achieved in eliminating pollen production in trees. One method by which pollen elimination could potentially be effected is by genetic ablation, the expression of a cytotoxic gene under the control of a tightly regulated promoter. The result of genetic ablation is the targeted elimination of specific cells or tissues of living organisms without lethal effects. In theory, the functional destruction of the tapetum by means of genetic ablation could reduce pollen production and release. The *PrMC2* gene, a male cone-specific promoter of radiata pine (*Pinus radiata*), encodes a protein similar to the anther-specific A9 protein in *Brassica napus* and Arabidopsis. Both are heavily expressed in the tapetum of the microsporangia. One of the contributions of the tapetum to the development of mature microspores is the release of enzymes that degrade the callose and pectic molecules to free individual microspores from the tetrad. In this issue, **Zhang et al. (pp. 1428–1439)** report upon the use of *PrMC2* to drive modified barnase (an RNase) coding sequences to determine their effectiveness in pollen ablation. An expression cassette consisting of *PrMC2* and one of the modified barnase sequences (H102E) was found to efficiently ablate pollen in tobacco (*Nicotiana tabacum*), pine, and eucalyptus (*Eucalyptus occidentalis*). Large-scale and multiple-year field tests demonstrated that complete prevention of pollen production was achieved in greater than 95% of independently transformed lines of pine and eucalyptus that contained the *PrMC2-barnaseH102E* expression cassette. This pollen-deficient phenotype was expressed stably over multiple years, multiple test locations, and when the *PrMC2-barnaseH102E* cassette was flanked by different genes. Except for their pollenless phenotype, the *PrMC2-barnaseH102E* transgenic pine and eucalyptus trees appear to grow similarly to control trees in all respects. This is not the first time that this general approach toward effecting pollen ablation has been attempted, but the transgenic plants reported upon earlier had negative attributes stemming from actions of the *barnase* gene on plant regeneration and vegetative growth that were probably associated with low levels of promoter activity in nontarget tissues. The stable and efficient pollen ablation performance of *PrMC2-barnaseH102E* under field conditions, achieved without any concomitant detrimental vegetative effects, makes this expression cassette useful in field plantings of transgenic trees when mitigation of pollen-mediated transgene flow is desired.

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