

Stress and innate immune responses in plants

Plant-pathogen interactions and response

Plant protein sentinels detect factors of pathogen origin. These factors likely play a role in aiding pathogen colonization of the host. Types of plant sentinels are proteins composed of motifs found in animal innate immune genes including Toll IL-1R-like or Coiled-coil domains juxtaposed to nucleotide binding domains and leucine rich repeats (LRR). There are hundreds of such genes in the plant genome implying a high degree of diversification and specificity (Pan et al., 2000 a,b; Sela-Buurlage et al., 2001; Kaplan-Levy and Fluhr, 2002). On the pathogen side, there is rapid adaptation to the changing circumstance of infection. Thus, in the case of pathogenic fungi causing disease in vascular tissue *Fusarium oxysporum* or *Colletotrichum coccodes* on tomato fruit, rapid pathogen adjustment to limiting host nutrients was shown to be essential for successful colonization (Divon et al., 2005, 2006, 2007; Alkan, 2008). As illustrated in Figure 1, the selection of mutants in the control of nitrogen metabolism leads to impairment of fungal pathogenicity.

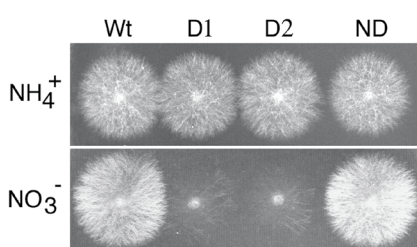


Fig. 1. Growth of *fnr1*- mutants of *Fusarium oxysporum* on media that is selective for nitrate reductase activity. The radial growth of wild-type and disruption mutants D1 and D2 is shown. Fungi that cannot regulate their growth on nitrate show aberrant pathogenicity characteristics (adapted from Divon 2006).

Protease and immune defense pathways

Activation of defense responses in animals involves protease pathways that are regulated by serpins (serine protease inhibitors). Serpins form suicide substrates with their cognate substrate.

We can show that serpin homologs exist in plants and play a role in the plant defense and wound response by directly regulating protease cascades. Loss and gain-of-function mutants of a serpin modifies important hormonal defense pathways controlled by salicylic and jasmonic acid suggesting a novel junction for hormonal cross-talk that fine-tunes environmental and defense responses (Bowler and Fluhr, 2000; Budai-Hadrian et al., 2008). One of the common and conserved elements in the innate immune molecules is the TIR (Toll/interleukin-1 receptor) domain. It plays a critical role in immunity and inflammation responses of mammals with similar components in *Drosophila*. In *Arabidopsis*, a group of resistance genes (R-genes) that can initiate hypersensitive response contain TIR motifs. Bioinformatic analysis of the *Arabidopsis* genome revealed a small gene family containing a unique combination of TIR and polysaccharide binding motifs that has a function in modulating the wound response (Zohar, 2008; PhD thesis). These findings point to diverse roles that the TIR-containing gene families can play in *Arabidopsis* innate immunity and general defense.

NADPH oxidases as signal transponders in pathogen and environmental stress

Plant NADPH oxidases are homologs of the human neutrophil pathogen-related gp91^{phox} and produce reactive oxygen species (ROS). Their activity

is rapidly induced by contact with pathogens (Allan et al., 2001) or elicitors. By employing antisense technology, tomato plants with diminished Rboh activity were produced that showed developmental changes and were compromised in wound-induced gene expression (Sagi et al., 2004). Extending these observations by microarray transcriptome analysis, revealed both Rboh-dependent and independent wound-induced gene induction pathways implying a role for Rboh as hormone signaling transponder (Sagi and Fluhr, 2006). We have examined very early events in cryptogei-induced ROS production in tobacco BY-2 suspension cells and show that unexpectedly the internal signal for H₂O₂ develops more rapidly than the external apoplasmic signal. Major accumulation of ROS was detected in endomembrane, cytoplasmic and nuclear compartments (Figure 2; Ashtamker et al 2007). The unexpected spatial and temporal appearance of the ROS signal implies a

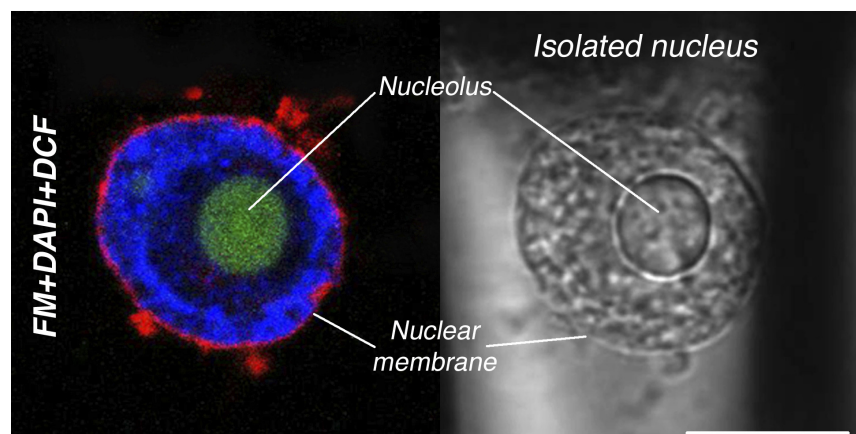


Fig. 2. Imaging of H₂O₂ signal in isolated nuclei in response to calcium application, as reported by DCF. The merged optical section of an isolated BY-2 nucleus is triple-stained with FM 4-64, DAPI and DCF (adapted from Ashtamker et al 2007).

complex role in cellular signaling.

Dynamics of alternative splicing in the plant genome

Adding to the complexity of resistance-gene biology are reports that implicate alternative splicing in R-gene function. In an effort to understand the relationship of alternative splicing to the dynamics of resistance and adaptation to stress in general, we have established a database of Arabidopsis splicing variants and have shown their regulation by specific kinases and by environmental stress (Savaldi-Goldstein et al., 2000, 2003). We were the first to show the prevalence of short intron types in Arabidopsis and to show that retained introns is the major splice variant (Ner-Gaon et al., 2004). We then went on to describe alternative splicing distribution in dicot and monocot species and showed the dependence of alternative splicing rate on genome size (Figure 3; Ner-Gaon et al., 2007). The results imply an evolutionary relationship between the two. Our continued interest is in developing tiling microarray technology so as to follow the dynamics of alternative splicing during stress and development (Ner-Gaon and Fluhr, 2006).

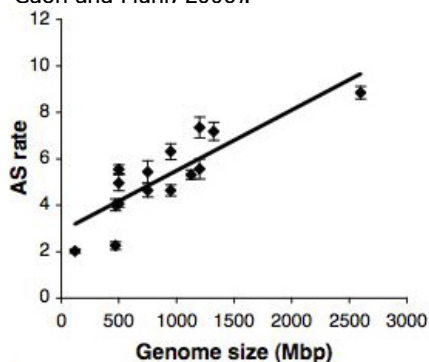


Fig. 3. AS rate as a function of EST sampling size and genome size in eudicot species. Randomly selected 20,000 EST of each species of *Arabidopsis thaliana* (Columbia), *Medicago truncatula* (a17), *Solanum lycopersicum* (ta496), *Oryza sativa* (nipponbare) and *Zea mays* (b73) are shown. The AS were normalized to the number of clusters containing more than 1 EST. Results are an average of the recurrent measurements plotted as a function of genome size (adapted from Ner-Gaon et al., 2007).

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