Spatial and temporal analysis of the local response to wounding in *Arabidopsis* leaves

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**Abstract**

We studied the local response to wounding in *Arabidopsis thaliana* leaves using a two-step microarray analysis. A microarray containing 3500 cDNA clones was first screened to enrich for genes affected by wounding in the immediate vicinity of the wound (4 h post wounding). 359 non-redundant putative wound responsive genes were then spotted on a smaller wound-response array for detailed analysis of spatial expression (local, adjacent and systemic), timing of expression (0.5, 4, 8, 17 h), and effect of hormone treatments (methyl jasmonate, ethylene and abscisic acid). Our results show that genes that respond early at the site of the wound also respond throughout the plant, with similar kinetics. Early-induced genes which respond systemically encode predominantly signal transduction and regulatory factors (36%), and the expression of many of them is also controlled by methyl jasmonate (about 35% of the 36%). Genes specific to the wound site and the wounded leaf have a slower response to wounding and are mainly metabolic genes. At the wound, many genes of the lignin biosynthesis pathway were induced. *In silico* analysis of the 5’ promoter regions of genes affected by wounding revealed G-box-related motifs in a significant proportion of the promoters. These results show that the establishment of a systemic response to wounding is a priority for the plant, and that the local response at the wound site is established later. Ethylene and abscisic acid are involved in the local response, regulating repression of photosynthetic genes and expression of drought responsive genes respectively.

**Introduction**

As sessile organisms, plants are forced to react to a number of biotic and abiotic stresses. Agents that inflict wounding and immediate tissue damage to the plant, such as herbivore feeding and adverse weather conditions, endanger plant survival by exposing the plant to water loss and further invasion by pathogens. The response of plants to mechanical wounding has been the subject of extensive investigations (for reviews, see: Bowles, 1990; Ryan, 2000; de Bruxelles and Roberts, 2001; Leon *et al.*, 2001). Some plant species have developed complex natural defense mechanisms to protect against wounding, ranging from physical barriers, including cuticle formation, lignification, thorns and trichomes (Levin, 1973), to the production of toxic components such as alkaloids (Baldwin, 1989) and tannins (Scalbert, 1991).

The wounding response (WR) involves repair of damaged wound tissue, production of compounds that limit herbivore feeding (e.g., proteinase inhibitors that limit digestibility; Peña-Cortés *et al.*, 1995), protection against subsequent infec-
tion by opportunistic pathogens, and the adjustment of plant metabolism to cope with all these changes. The WR can be subdivided into a local response, occurring in the immediate vicinity of the wound, and a systemic response, occurring throughout the plant (Bowles, 1993). Genes involved in the local response are predicted to play a role in wound healing and repair, as well as protection against water loss and invasion of pathogens. The systemic genes produce a defense mechanism against further attack by herbivores or pathogens.

Recently, microarrays have enabled the large-scale identification of WR genes and the functional dissection of the response. A substantial overlap was demonstrated between wounding and the response to water stress (Reymond et al., 2000), and between wounding, pathogen response and other signaling pathways (Cheong et al., 2002). The finding that the ADC2 (arginine decarboxylase) gene is induced by wounding, suggested an involvement of polyamines in the response (Perez-Amador, 2002).

The systemically induced genes and the long-distance signaling aspects of the WR have been the subject of extensive investigations. Several signaling mechanisms have been identified (for review, see: Ryan, 2000; de Bruxelles and Roberts, 2001; Leon et al., 2001). Oligogalacturonides derived from damaged plant cell walls or from fungal pathogens induce proteinase inhibitor (pin) genes in tomato (Doares et al., 1995; Bergey et al., 1999). The mobility of oligogalacturonides is limited and they are involved in the local response to wounding. In solanaceous plants, the 18 amino acid peptide systemin was shown to function as a long distance signal (Ryan, 2000). Systemin activates a lipid-derived signalling pathway, leading to the local and systemic accumulation of the hormone jasmonic acid (JA) and its ester methyl jasmonate (MeJA; Farmer and Ryan, 1990). More recently, reciprocal grafting experiments demonstrated that activation of the JA biosynthetic pathway by wounding or systemin is required for the production of a long-distance signal whose perception in distal leaves depends on JA signaling, suggesting that JA or a related compound of the octadecanoid pathway may act as a transmissible wound signal (Li et al., 2002; Lee and Howe, 2003). However, in systemic leaves, accumulation of JA or its bioactive precursor OPDA (12-oxophytodienoic acid) could not be detected even though activation of defense gene expression occurred (Strassner et al., 2002). Wounded plants also accumulate abscisic acid (ABA) in the region surrounding the wound site (Peña-Cortés et al., 1995; Birkenmeier and Ryan, 1998). ABA may function in a dehydration response pathway at the wound site (Reymond et al., 2000). Wounding also induces ethylene biosynthesis and ethylene acts in conjunction with JA to regulate pin expression (O’Donnell et al., 1996). In addition to these signals, the WR was shown to involve reactive oxygen species (ROS; Orozco-Cardenas and Ryan, 1999), secondary messengers (e.g., calcium), and phosphorylation events (for review, see Leon et al., 2001).

In order to study which factors are involved in the establishment and regulation of the WR we focused our microarray approach on the analysis of gene expression at the wound site. To achieve this, we used a two-step microarray approach. We enriched for genes expressed at the wound site in the first step, and then printed these genes on a smaller wound responsive (WR) array. On this specialized array we carried out a detailed study of spatial and temporal expression, and response to phytohormones of WR genes. Our results show that the systemic response is established earlier than the local response, involves mainly signaling and regulatory factors, and is partly regulated by MeJA. Genes that are specific to the local response encode mainly proteins with metabolic functions.

**Materials and methods**

**Plant material**

*Arabidopsis thaliana* ecotype C24 plants were grown in 80mm petri dishes on Murashige–Skoog (MS) medium containing 3% sucrose and 0.8% agar, under 16 h day-light (75–100 μmole/m²/s) at 22 °C. After a week, the plantlets were transferred to new petri dishes containing MS-agar medium (3% sucrose), at a density of 5 plantlets/dish to improve growth and expansion of the leaves. In the case of plants used in phytohormone treatments, the plants were grown under the same conditions and were transferred to petri dishes containing 15 ml of liquid MS medium (3% sucrose) 3 days prior to the start of the treatment.