Showcase on Research

Transcriptional Control of Plant Defence Gene Expression

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Plant diseases cause major losses in productivity worldwide. The losses are often around 20% in developed nations and 30-45% in developing nations (1). Understanding how plants defend themselves against a range of pathogens is therefore of critical importance for successful agriculture. The major groups of plant pathogens and pests are bacteria, fungi, insects, nematodes and viruses. Plants protect themselves through a variety of means, including preformed structural defences that act as physical barriers and the constitutive expression of secondary metabolites with antimicrobial activity.

Plants are also capable of activating inducible defence responses following pathogen perception. The speed with which these defence mechanisms are deployed is often a crucial factor in successful resistance. Pathogen perception can be regulated by a gene-for-gene interaction involving plant resistance genes (R) and corresponding pathogen avirulence genes (*avr*) (reviewed in ref. 2).

A hallmark of the gene-for-gene interaction is the high degree of specificity that can often occur whereby different cultivars of a plant species are resistant only to specific races of a pathogen. R gene activation leads to a number of changes that occur both at the infection site and systemically throughout the plant and include cell wall fortifications and enhanced defence gene expression. The localised response, known as the hypersensitive response (HR), leads to cell death to prevent pathogen spread. The systemic response, known as systemic acquired resistance (SAR), can last for several weeks and helps both to prevent the initial infection from spreading and to combat secondary infections by a broad range of pathogens.

Plant defence genes

A key component for effective plant resistance is the ability to rapidly induce a battery of plant defence genes. A major group of defence genes are the pathogenesis-related (PR) genes. While the function of many PR genes remains to be determined some are chitinases and glucanases which attack components of fungal cell walls.

Other defence genes activated include defensins, enzymes involved in phytoalexin biosynthesis, plant protectant enzymes and signal transduction components including specific transcription factors. Defence gene induction occurs primarily at the level of transcription and regulating the temporal and spatial expression patterns of specific defence genes is an important part of the plant defence response.

While individual PR genes have been overexpressed in transgenic plants, this approach has had limited success in increasing disease resistance. There is interest in identifying signal transduction components important for plant defence gene expression, including key transcription factor combinations, in the hope they may provide tools to enhance disease resistance to a range of pathogens by controlling the expression of suites of defence genes. Breakthroughs in this rapidly moving research area are often occurring in the model organism Arabidopsis, with the goal of moving results obtained in Arabidopsis into crop plants using recombinant DNA techniques.

Signalling pathways in plant defence gene expression

Defence responses are regulated by multiple defence signalling molecules including salicylic acid (SA), ethylene, nitric oxide (NO), jasmonic acid (JA) and reactive oxygen species (ROS) such as hydrogen peroxide (reviewed in refs 3, 4). ROS and NO are important signalling molecules during the HR and appear to work together to bring about localised plant cell death. SA levels rise significantly at the site of infection and to a lesser extent elsewhere in the plant and exogenous SA treatment enhances resistance to many pathogens. Transgenic plants unable to accumulate SA, due to the presence of a bacterial enzyme that converts SA to an inactive form, do not develop SAR and the induction of defence gene expression is abolished.

While SA has been shown to be important for defence against a range of pathogens including viruses, bacteria and biotrophic fungi, defence responses against necrotrophic fungi are likely to be mediated primarily by ethylene and JA signalling pathways working synergistically. Genetic approaches are identifying many of the components of the signalling pathways mediated by SA and ethylene/JA and a few key mutants are shown in **Fig. 1**.

There is significant overlap between the

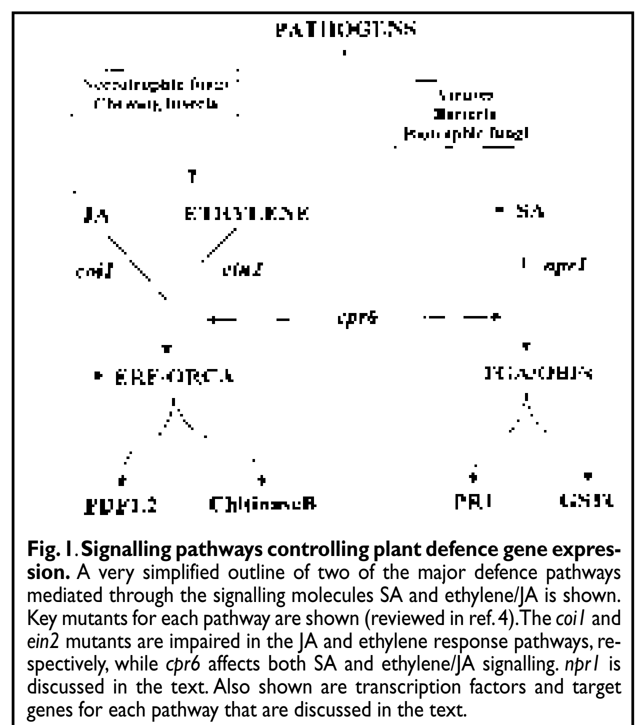

Fig. 1. Signalling pathways controlling plant defence gene expression. A very simplified outline of two of the major defence pathways mediated through the signalling molecules SA and ethylene/JA is shown. Key mutants for each pathway are shown (reviewed in ref. 4). The *coi1* and *ein2* mutants are impaired in the JA and ethylene response pathways, respectively, while *cpr6* affects both SA and ethylene/JA signalling. *npr1* is discussed in the text. Also shown are transcription factors and target genes for each pathway that are discussed in the text.

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patterns of plant gene expression induced in response to biotic stress and specific abiotic stresses. What once appeared to be a number of relatively simple linear pathways are now being revealed as a complex regulatory network of signals that allows plants to optimally respond to the changing environment they live in.

For the remainder of this review we will concentrate on the transcriptional control of plant defence gene expression and outline some future research directions that are likely to be productive. We will focus on two groups of plant transcription factors that have been strongly linked to defence responses and which are being actively pursued in our group.

Other transcription factors have also been implicated in plant defence gene expression including WRKY proteins, a novel family of transcription factors unique to plants. Specific WRKY family members show enhanced expression and/or DNA binding activity following induction by a range of pathogens and defence signals (5).

TGA/OBF transcription factors and SA signalling

One class of plant transcription factors that have been strongly linked to defence expression is the TGA/OBF (ocs-element binding factor) family. These proteins belong to a specific class of bZIP transcription factors that bind to a well-characterised plant promoter sequence called the ocs element (6). Ocs elements, also known as as-1-like elements, were initially identified in pathogen promoters where they have been exploited to express pathogen genes in plants.

Ocs elements are responsive to two key plant defence signals, SA and hydrogen peroxide, and regulate the expression of several genes likely to be involved in defence responses including the Arabidopsis PR-1 and glutathione *S*-transferase 6 (GST6) genes (7, 8). Recently TGA/OBF family members have been found to interact specifically with NPR1, a key component in the SA defence signalling pathway (9). *npr1* mutant plants are more susceptible to infection and do not express SA-regulated PR genes.

There are seven Arabidopsis members of the TGA/OBF family. Individual proteins vary in their DNA binding specificity, protein-protein interaction properties and expression patterns. For example, only some members interact with NPR1. As shown in **Fig. 2A**, TGA/OBF proteins bind as dimers to each half of the ocs element resulting in a functional tetramer.

Since the seven family members are able to form heterodimers the number of possible combinations of tetramers that can occupy the ocs element is large. This complexity may help explain how the ocs element is able to respond to a number of different signals including defence signals, xenobiotics and heavy metals **(Fig. 2A)**, and emphasises the overlap between biotic and abiotic stress responses.

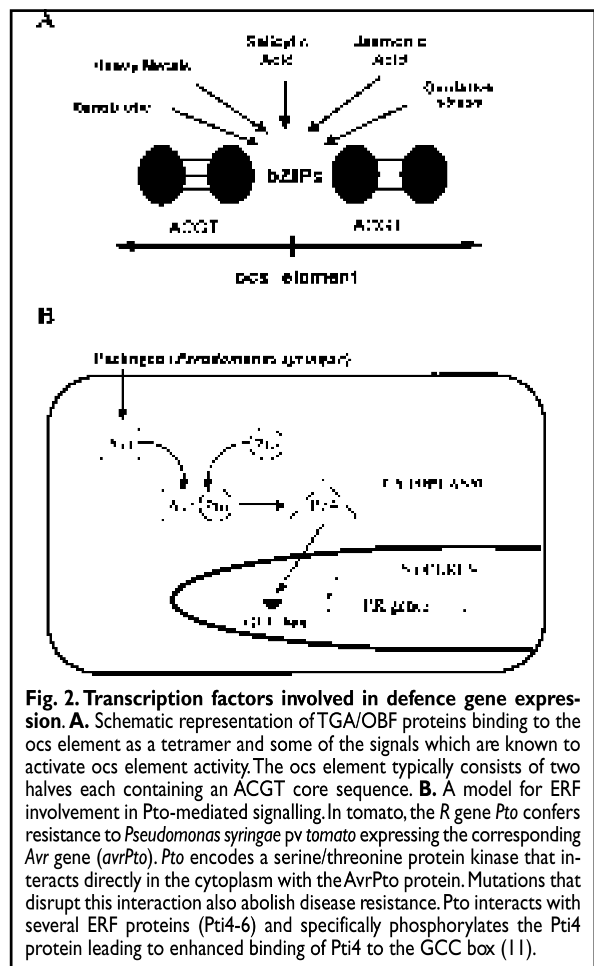

Fig. 2. Transcription factors involved in defence gene expression. A. Schematic representation of TGA/OBF proteins binding to the ocs element as a tetramer and some of the signals which are known to activate ocs element activity. The ocs element typically consists of two halves each containing an ACGT core sequence. **B.** A model for ERF involvement in Pto-mediated signalling. In tomato, the *R* gene *Pto* confers resistance to *Pseudomonas syringae* pv *tomato* expressing the corresponding *Avr* gene (*avrPto*). *Pto* encodes a serine/threonine protein kinase that interacts directly in the cytoplasm with the AvrPto protein. Mutations that disrupt this interaction also abolish disease resistance. Pto interacts with several ERF proteins (Pti4-6) and specifically phosphorylates the Pti4 protein leading to enhanced binding of Pti4 to the GCC box (11).

ERF/ORCA transcription factors and ethylene/JA signalling

ERF (ethylene-responsive element binding factors) proteins are novel plant transcription factors, many of which have been strongly linked to plant stress responses. The first family members were isolated through their binding to a *cis*-element known as the GCC box (reviewed in ref. 10), an 11-bp conserved sequence (TAAGAGCCGCC) initially found in several PR gene promoters that confers ethylene responsiveness. ERF proteins share a well-conserved 58-59 amino acid domain, the ERF domain, that has a novel structure thus far only found in plants.

Several ERF proteins (Pti4-6) were identified by their interaction with Pto, the product of the tomato *R* gene conferring resistance to the pathogen *Pseudomonas syringae*. The Pto protein is a serine/threonine kinase that can phosphorylate Pti4 thereby increasing its DNA binding to the GCC box (11). This leads to what may be a relatively simple signal transduction pathway as outlined in **Fig. 2B**.

In Arabidopsis, another ERF protein, AtEBP, was isolated by its interaction with a TGA/OBF protein (12). Recently, AtEBP expression was shown to be locally downregulated at the syncytium in a compatible cyst nematode infection, raising the possibility that suppression of AtEBP expression is involved in allowing a successful nematode infection (13). Further evidence linking ERF proteins to plant defence gene expression comes from reverse genetic studies where over-expression of the Arabidopsis ERF1 protein enhanced expression of a defensin (PDF1.2) and a chitinase gene (14).

In periwinkle, fungal elicitor and/or jasmonate inducible ERF domain-containing proteins named ORCAs (octadecanoid-derivative responsive *Catharanthus* AP2-domain) have been identified (15). An important JA-regulated defence response is the biosynthesis of protective secondary metabolites. ORCAs provide a link between JA and the production of secondary metabolites for defence, as overexpression of ORCA3 results in enhanced expression of several metabolite

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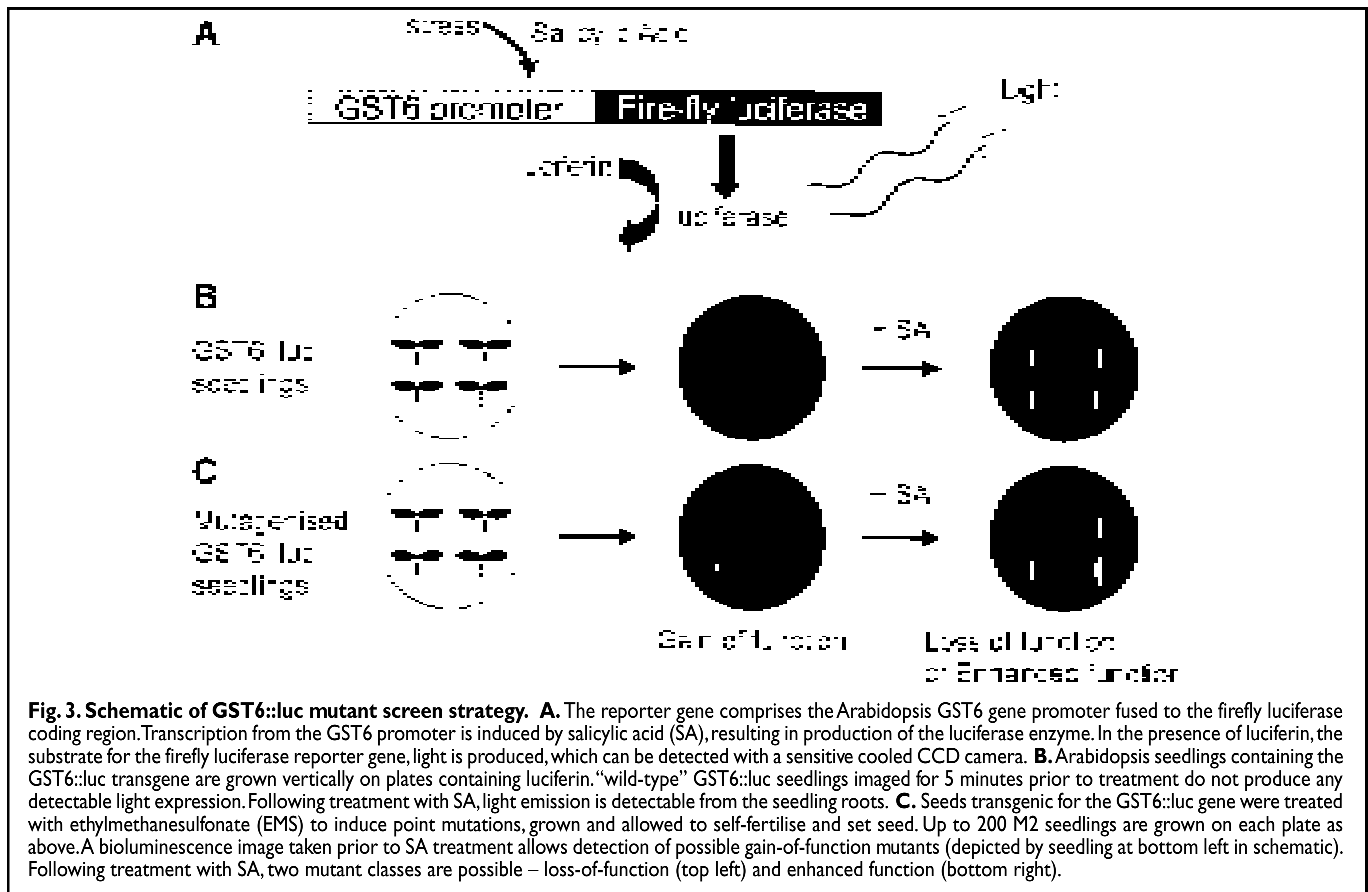

Fig. 3. Schematic of GST6::luc mutant screen strategy. A. The reporter gene comprises the Arabidopsis GST6 gene promoter fused to the firefly luciferase coding region. Transcription from the GST6 promoter is induced by salicylic acid (SA), resulting in production of the luciferase enzyme. In the presence of luciferin, the substrate for the firefly luciferase reporter gene, light is produced, which can be detected with a sensitive cooled CCD camera. **B.** Arabidopsis seedlings containing the GST6::luc transgene are grown vertically on plates containing luciferin. "wild-type" GST6::luc seedlings imaged for 5 minutes prior to treatment do not produce any detectable light expression. Following treatment with SA, light emission is detectable from the seedling roots. **C.** Seeds transgenic for the GST6::luc gene were treated with ethylmethanesulfonate (EMS) to induce point mutations, grown and allowed to self-fertilise and set seed. Up to 200 M2 seedlings are grown on each plate as above. A bioluminescence image taken prior to SA treatment allows detection of possible gain-of-function mutants (depicted by seedling at bottom left in schematic). Following treatment with SA, two mutant classes are possible – loss-of-function (top left) and enhanced function (bottom right).

biosynthetic genes through a GCC box sequence, resulting in increased accumulation of terpenoid indole alkaloids.

Some ERF proteins are also involved in responses to abiotic stresses (reviewed in ref. 16). For example, CBF and DREB proteins bind to a *cis*-element resembling the GCC box and their expression is upregulated by cold and dehydration, respectively. Overexpression of CBF1 confers cold tolerance in Arabidopsis, providing an excellent example of how a single regulatory gene can have a major impact on a complex plant stress response.

Future directions

While many of the key factors in plant defence signalling have been identified, there is still much information to be elucidated. The power of molecular genetics in model organisms is being brought to bear on the problem in a number of ways. For example, reverse genetics offers increasingly powerful approaches to determine the function of already identified genes. Moreover, the coupling of reverse genetics and DNA microarrays for genome wide analysis of gene expression patterns (17) will help allow the role of specific transcription factors to be investigated by determining the complete network of genes influenced in specific gain- or loss-of-function mutants.

Traditional forward genetic approaches, which aim to identify a gene defined by a mutant phenotype, still have much to offer. The key to a successful mutant screen is an easily determined phenotype allowing high throughput screening. By utilising an easily assayed reporter gene to reflect the overall status of a plant, researchers can avoid direct but laborious screens, such as for enhanced resistance to a fungal pathogen. The firefly luciferase (LUC) reporter gene has been successfully exploited for the identification of circadian rhythm mutants (18) as well as for mutants altered in osmotic and cold stress signalling (19).

LUC has a distinct advantage over some other reporters in that real-time expression can be monitored non-invasively using a cooled CCD camera system, allowing a large population to be screened for mutants that can then be recovered and propagated.

We are using a stress-responsive promoter fused to LUC to isolate mutants in stress signalling pathways. We have fused our well-characterised GST6 promoter, which contains an ocs element and responds to salicylic acid as well as a number of other stress signals predominantly in roots (8), to the LUC coding region. We have generated transgenic plants where luciferase expression accurately reflects endogenous GST6 regulation (see cover). Thus, we can monitor expression of the GST6::LUC transgene to gauge the stress status of these plants.

A population homozygous for the GST6::LUC transgene was treated with the mutagen EMS. The subsequent generation is being used to screen young seedlings for both loss- and gain-of-function mutations in the salicylic acid signalling pathway **(Fig. 3)**. We are optimistic that the non-invasive, high throughput nature of the screen will contribute in particular to our understanding of stress responses in roots. This is an area where much less is known than for foliar diseases, stemming in part from the difficulties in directly observing roots.

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Concluding remarks

The importance of identifying key regulatory factors for improving specific plant traits through genetic engineering is well illustrated by work on NPR1. Overexpression of NPR1 in Arabidopsis results in stronger defence gene expression and confers enhanced disease resistance to both a bacterial and fungal pathogen without producing deleterious effects, thereby demonstrating the potential for single regulatory genes to provide broad spectrum resistance (20).

The array of powerful genetic approaches being deployed should continue to provide candidates for engineering enhanced disease responses in plants in the continuing battle to keep plants one step ahead of the pathogens.

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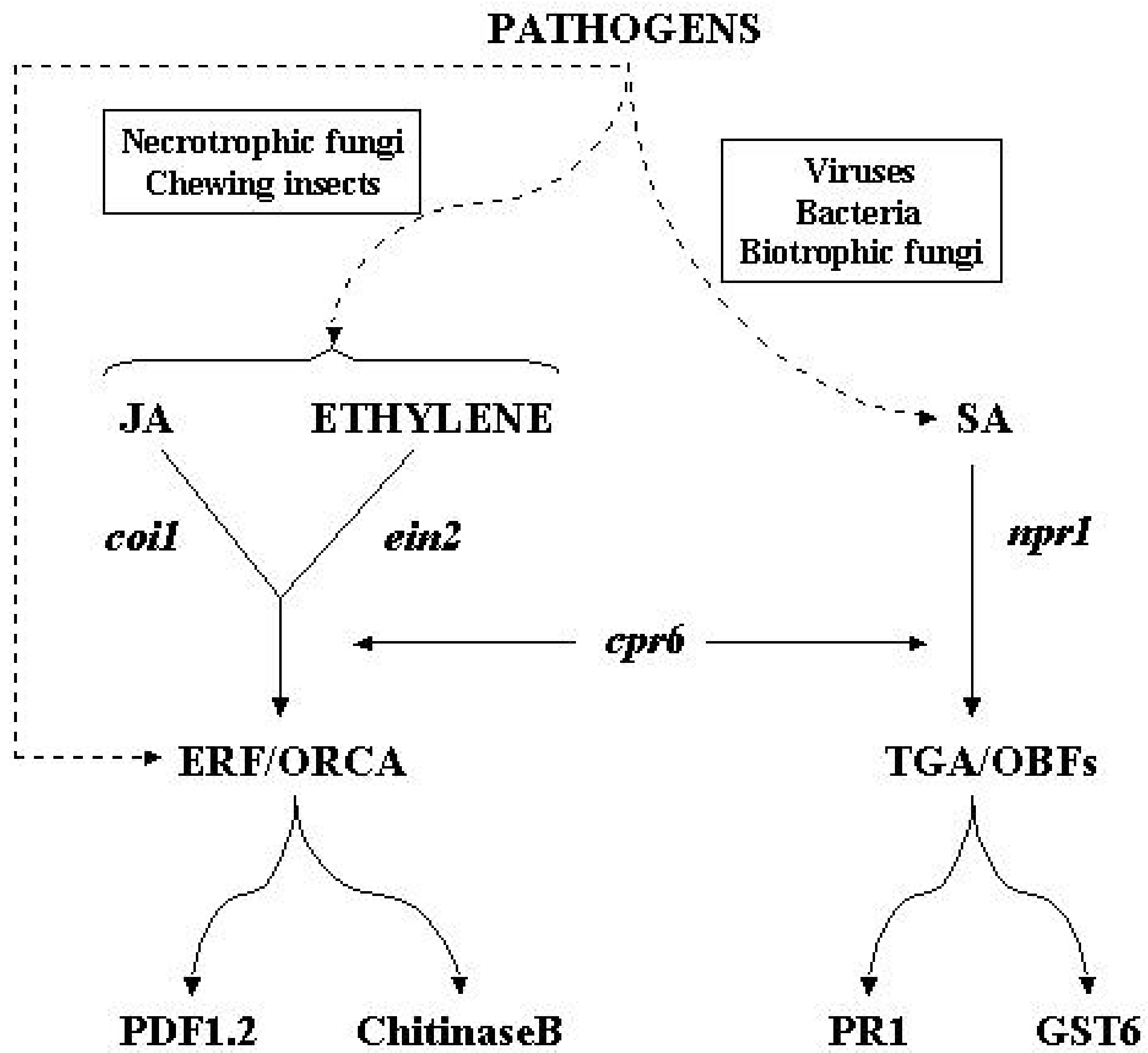
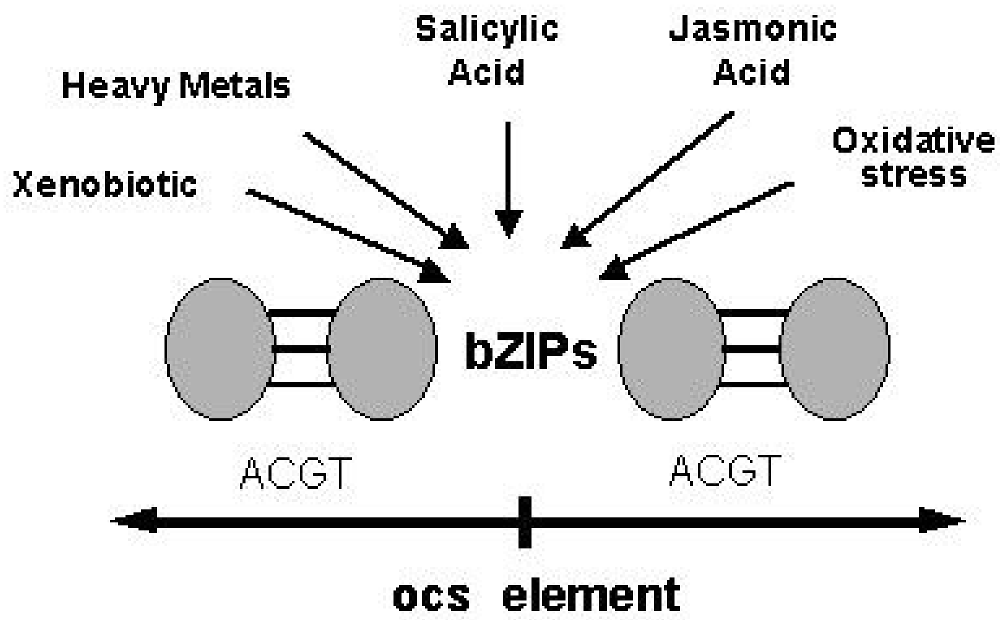


Fig. 1



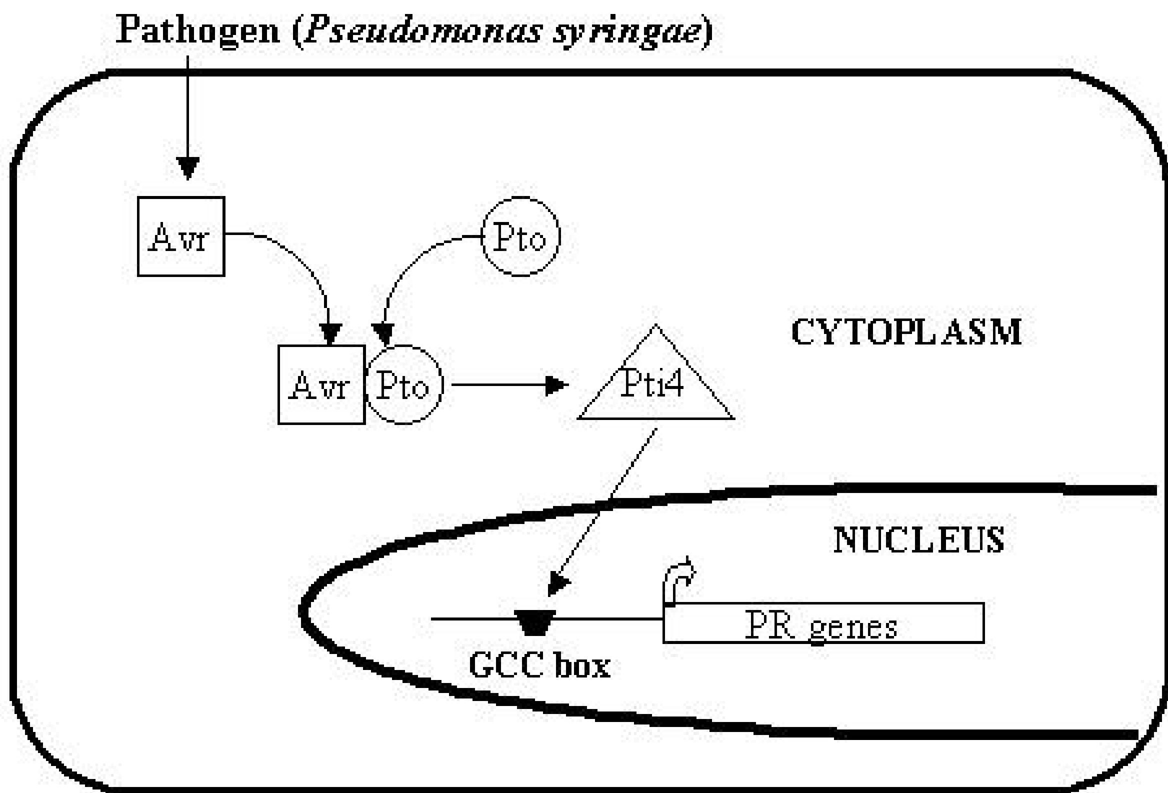


Fig. 2

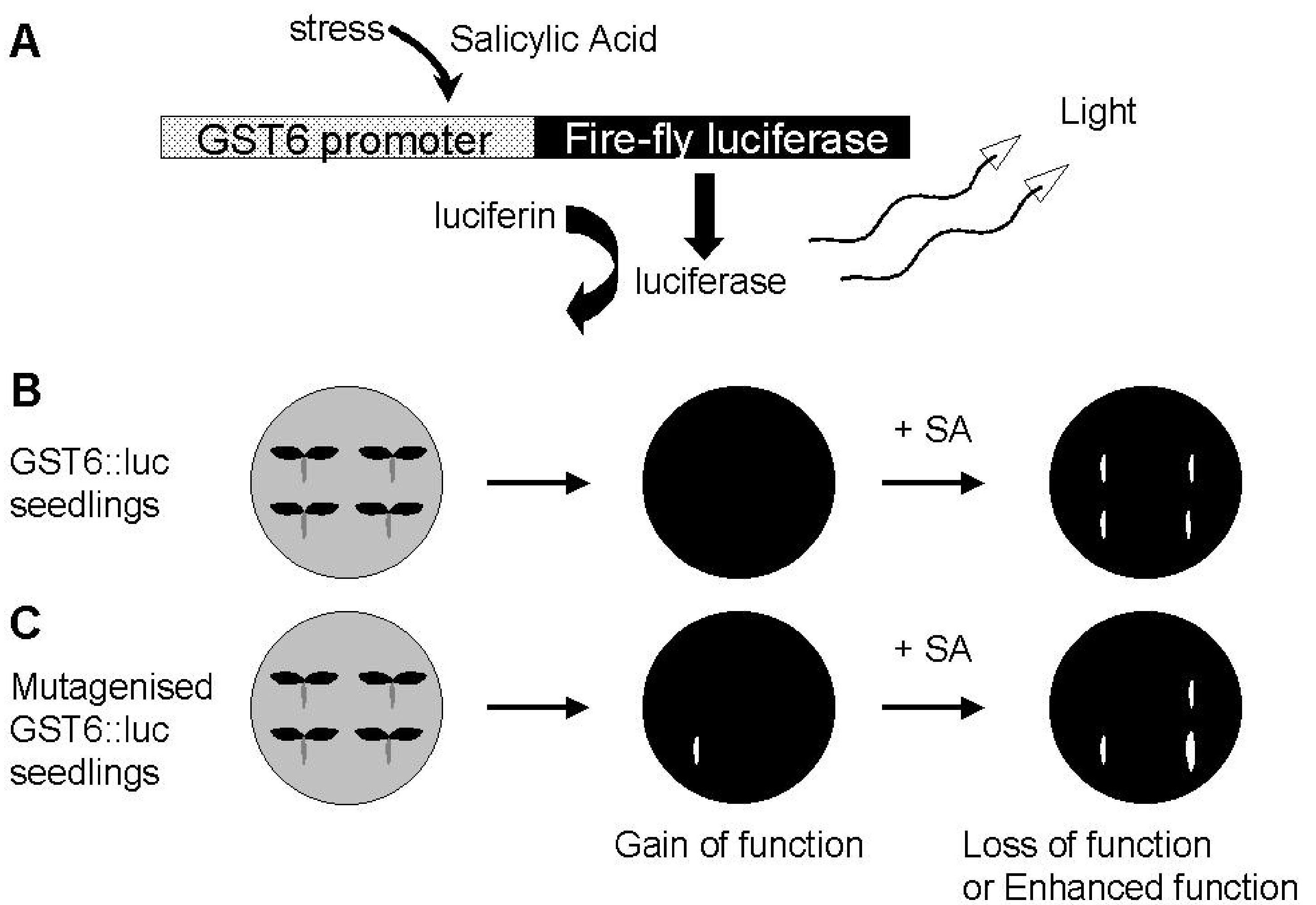


Fig. 3