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Nucleotide sequence of a pathogen induced nitrilase gene from *Arabidopsis thaliana* : Nit2 (Accession No. [U47114](#))

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Nitrile aminohydrolase (Nitrilase; EC [3.5.5.1](#)) is the enzyme that converts IAN into IAA in *Arabidopsis thaliana* (Bartling *et al.* , 1994). Thus, understanding the regulation of nitrilase will be instrumental in understanding IAA biosynthesis and its regulation at the molecular level.

Nitrilase cDNAs (Bartling *et al.* , 1992, 1994; Bartel and Fink, 1994) and genes (Bartel and Fink, 1994) have been cloned from *Arabidopsis*. The NIT1 and NIT2 genes are adjacent on chromosome III (Bartel and Fink, 1994), yet they are differentially regulated. While NIT1 is expressed throughout development, NIT2 is most strongly expressed during silique development (Bartling *et al.* , 1994). In addition, NIT2 is highly expressed after bacterial infiltration with *Pseudomonas syringae* pv. *maculicola* (Bartel and Fink, 1994). The presumed result of this would be increased conversion of IAN to IAA after bacterial infection. Indeed, increases in IAA content of tobacco tissues after infection with *P. solanacearum* have been previously observed (Maine, 1960; Sequiera, 1965).

On the basis of earlier sequencing runs, primers were designed to extend the new sequence. DNA sequences were performed in duplicate for each run. Sequencing reactions were performed using the Applied Biosystems Prism Dye-deoxy Cycle Sequencing Kit. The reactions were run on an Applied Biosystems Prism 377 DNA sequencer, Perkin-Elmer Corp. The sequence of the Nitrilase 2 gene was extended from the coding region of the Nitrilase 2 gene for approximately 1.8 kb. Then the entire sequence was confirmed on the opposite strand.

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Table 1. Characteristics of the Nitrilase 2 promoter from *Arabidopsis thaliana*

Organism:*Arabidopsis thaliana***Gene Product:**Nitrilase - nitrile aminohydrolase EC [3.5.5.1](#), catalyzes the conversion of IAN into IAA**Characteristics of the promoter:**

The sequenced DNA fragment of the Nitrilase 2 gene was 4374 nucleotides covering the entire Nitrilase 2 structural gene and 1774 nucleotides of the 5' upstream region. By matrix analysis, the sequence of the NIT2 promoter shows little identity with the NIT1 promoter (Zhou *et al.* , in press).

Upstream Region -- 1774 Nucleotides (1..1774)

Putative promoter elements:

Direct Repeats

2 non-identical direct repeats were identified

- 1) 81% identity -- (691..777) and (847..934)
- 2) 83% identity -- (943..1029) and (1035..1120)

Hairpin Loops

6 hairpin loops with stems greater than 10 bp were detected.

- 1) 13/14 nt in stem, 1 nt in loop -- (473..501)
- 2) 10/12 nt in stem, 10 nt in loop -- (584..617)
- 3) 12/14 nt in stem, 1 nt in loop -- (737..765)
- 4) 12/14 nt in stem, 2 nt in loop -- (1173..1202)
- 5) 13/14 nt in stem, 6 nt in loop -- (1202..1235)
- 6) 12/15 nt in stem, 0 nt in loop -- (1188..1217)

note: hairpin loops 4,5, & 6 are contained within the same nucleotide stretch and are incompatible with each other (either loops 4 and 5 can form, or loop 6 can form, but not both).

TATA box

(1723..1735) [8/13] to canonical plant TATA box (Joshi, 1987)

bZIP Binding Sites (ACGT)

175, 344

Additional Open Reading Frames

20 small open reading frames varying in size from 31 to 57 amino acids were identified in the intragenic region. BLAST searches (Altschul *et al.* , 1990) of these ORFs failed to identify homologous sequences in the databases.

5' end of Nitrilase 2 cDNA -- Nucleotide 1775

5' untranslated region -- 20 Nucleotides (1775..1794)

ATG start codon -- 1795

Intron 1 -- 71 Nucleotides (1905..1975)

Intron 2 -- 547 Nucleotides (2155..2701)

Intron 3 -- 92 Nucleotides (2966..3087)

Intron 4 -- 92 Nucleotides (3617..3861)

TAG stop codon -- 3726

3' untranslated region -- 245 Nucleotides (3729..3973)

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