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## Nucleotide sequence of the *Arabidopsis thaliana* nitrilase 1 gene (Accession No. **U38845**)

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Current analytical data suggests that there are multiple pathways for the biosynthesis of indoleacetic acid (IAA) in *A. thaliana* (Butcher *et al.* , 1974; Hogge *et al.* , 1988; Ludwig-Muller and Hilgenberg, 1988; Normanly *et al.* , 1993), yet *at* 1 these pathways involve indole acetonitrile (IAN) as the direct auxin precursor. Nitrilase (nitrile aminohydrolase; EC [3.5.5.1](#)) is the enzyme that converts IAN into IAA and must therefore likely to be one of the key enzymes in the biosynthesis of IAA (Bartling *et al.* , 1994). Thus, understanding the regulation of nitrilase will be instrumental to understand 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 cDNAs show strong homology to the bacterial nitrilase genes (Bartling *et al.* , 1992). When expressed in *Escherichia coli* , NIT1 and NIT2 cDNAs do indeed express functional nitrilase protein that can convert IAN into IAA (Bartling *et al.* , 1992, 1994). Subcellular localization of NIT1 and NIT2 indicates that NIT1 is soluble and is expressed throughout development, whereas NIT2 is membrane bound and is most strongly expressed during silique development (Bartling *et al.* , 1994). Gene analysis indicates that there is a small gene family with four gene members, three of which (NIT1, NIT2, and NIT3) are clustered on chromosome III and that NIT4 resides alone on chromosome V (Bartel and Fink, 1994). Northern analysis indicates that this gene family is differentially expressed: NIT1 being primarily expressed in the leaves with lower levels expressed in siliques, NIT2 being expressed principally in siliques, NIT3 being expressed predominantly in the roots, and NIT4 being expressed in siliques, stems, and leaves

(Bartel and Fink, 1994).

On the basis of earlier sequencing runs, primers were designed to extend 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 1 gene was extended on one strand until the 3' end of the Nitrilase 2 gene was identified. Then the entire sequence was confirmed on the opposite strand independently.

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### Table 1. Characteristics of the Nitrilase 1 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 1 gene was 3649 nucleotides covering the entire intragenic region between the Nitrilase 2 gene extending through the Nitrilase 1 structural gene.

Nitrilase 2 --

3' untranslated region -- 130 Nucleotides (1..130)

Intragenic Region -- 1466 Nucleotides (131..1596)

Putative promoter elements:

TATA box

1544..1555 (9/13) to canonical plant TATA box (Joshi, 1987)

Direct Repeats

1) 16 of 17 identical - 1141..1157 and 1168..1184

2) 16 of 17 identical - 1402..1418 and 1427..1443

Hairpin Loops

1) 1071-TATATAC-3 nt-GTATATA-1088

2) 1123-TATGGATT-5 nt-AATCCATA-1143

3) 1170-AAAAGAA-4 nt-TTCTTTT-1087

4) 1443-GTGGG-5 nt-CCCAC-1456

Palindromes (8 nt or greater)

1) 1025-TCGTACGA-1033

2) 1038-CCACGTGG-1046

3) 1602-ACTCGAGT-1610

bZIP Binding Sites (ACGT)

307, 376, 895, 1440, 1514

Additional Open Reading Frames

13 small open reading frames varying in size from 50 to

129 amino acids were identified in the intragenic region.

BLAST searches of these ORFs failed to identify any homologous sequences in the databases.

5' end of Nitrilase 1 cDNA -- Nucleotide 1597

5' untranslated region -- 17 Nucleotides (1597..1613)

ATG start codon -- 1614

Intron 1 -- 92 Nucleotides (1744..1835)

Intron 2 -- 629 Nucleotides (2016..2644)

Intron 3 -- 93 Nucleotides (2939..3031)  
Intron 4 -- 84 Nucleotides (3314..3397)  
TAG stop codon -- 3550  
3' untranslated region -- 97 Nucleotides (3553..3649)

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