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Sequence analysis of a chalcone isomerase cDNA of *Phaseolus vulgaris* L.

E.R. Blyden[1], P.W. Doerner[2], C.J. Lamb[2] and R.A. Dixon[3]*
[1]*Department of Genetics, Free University, De Boelelaan 1087, 1081 HV Amsterdam, Netherlands;* [2]*Salk Institute for Biological Studies, 10010 N. Torrey Pines Road, La Jolla, CA 92037, USA;* [3]*Plant Biology Division, Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402, USA (*author for correspondence).*

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GAATTCCTCCACTTCCCTGCGGTGGTTACTTCACCGGCCACTGCCAAAACCTATTTCCTC  60
 E  F  L  H  F  P  A  V  V  T  S  P  A  T  A  K  T  Y  F  L

GGCGGCGCAGGGGAGAGAGGATTGACGATTGAGGGGAAATTCATAAAGTTCACGGCCATC 120
 G  G  A  G  E  R  G  L  T  I  E  G  K  F  I  K  F  T  A  I

GGAGTATACTTGGAGGATAAAGCGGTGGCGTCACTCGCAACAAAGTGGAAGGGTAAGCCA 180
 G  V  Y  L  E  D  K  A  V  A  S  L  A  T  K  W  K  G  K  P

TCGGAAGAGTTGATCAACACTCTTGACTTCTACAGAGATATCATTTCAGGACCCTTTGAA 240
 S  E  E  L  I  N  T  L  D  F  Y  R  D  I  I  S  G  P  F  E

AAGCTAATAAGAGGTTCGAAGATTCTGCAATTGAGTGGGACAGAATACTCGAGGAAGGTG 300
 K  L  I  R  G  S  K  I  L  Q  L  S  G  T  E  Y  S  R  K  V

ATGGAAAACTGCGTGGCACACTTGAAGTCTGTTGGGACATATGGTGATGCTGAAGCCAAA 360
 M  E  N  C  V  A  H  L  K  S  V  G  T  Y  G  D  A  E  A  K

GGCATTGAAGAGTTTGCAGAAGCCTTCAAGAAAGTGAATTTTCCACCTGGTGCCTCTGTT 420
 G  I  E  E  F  A  E  A  F  K  K  V  N  F  P  P  G  A  S  V

TTCTACCGACAATCACCTGATGGAATCTTGGGGCTTAGTTTCTCTGAAGATGCAACGATA 480
 F  Y  R  Q  S  P  D  G  I  L  G  L  S  F  S  E  D  A  T  I

CCGGGAGAAGAGGCTGTAGTTATAGAGAACAAGGCTGTATCAGCTGCAGTATTGGAGACT 540
 P  G  E  E  A  V  V  I  E  N  K  A  V  S  A  A  V  L  E  T

ATGATCGGAGAACATGCTGTCTCCCCTGACTTGAAACGTAGTTTGGCTTCACGATTGCTG 600
 M  I  G  E  H  A  V  S  P  D  L  K  R  S  L  A  S  R  L  L

CGGTATTGAATGGCGGCATTATAGTCTGAAAATTGAGGAACACATGAGTTGAGGAAAATT 660
 R  Y

AGTGCATGAACTTGAGTTTTTCAAATATTATTGTTTTTCTCTCTTTCTTCTCACTAAAGT 720
TGTTAAACTTACTCATGTATTTCTACTTGTTTATCAAATAAATAAATAAAATCTGAGTTT 780
GAGATTTTCTTAAAAAAAAAAAAAAAA
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Fig. 1. The nucleotide and derived amino acid sequences of the bean CHI cDNA clone in pCHI1. Potential polyadenylation signals are underlined.

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X16470.

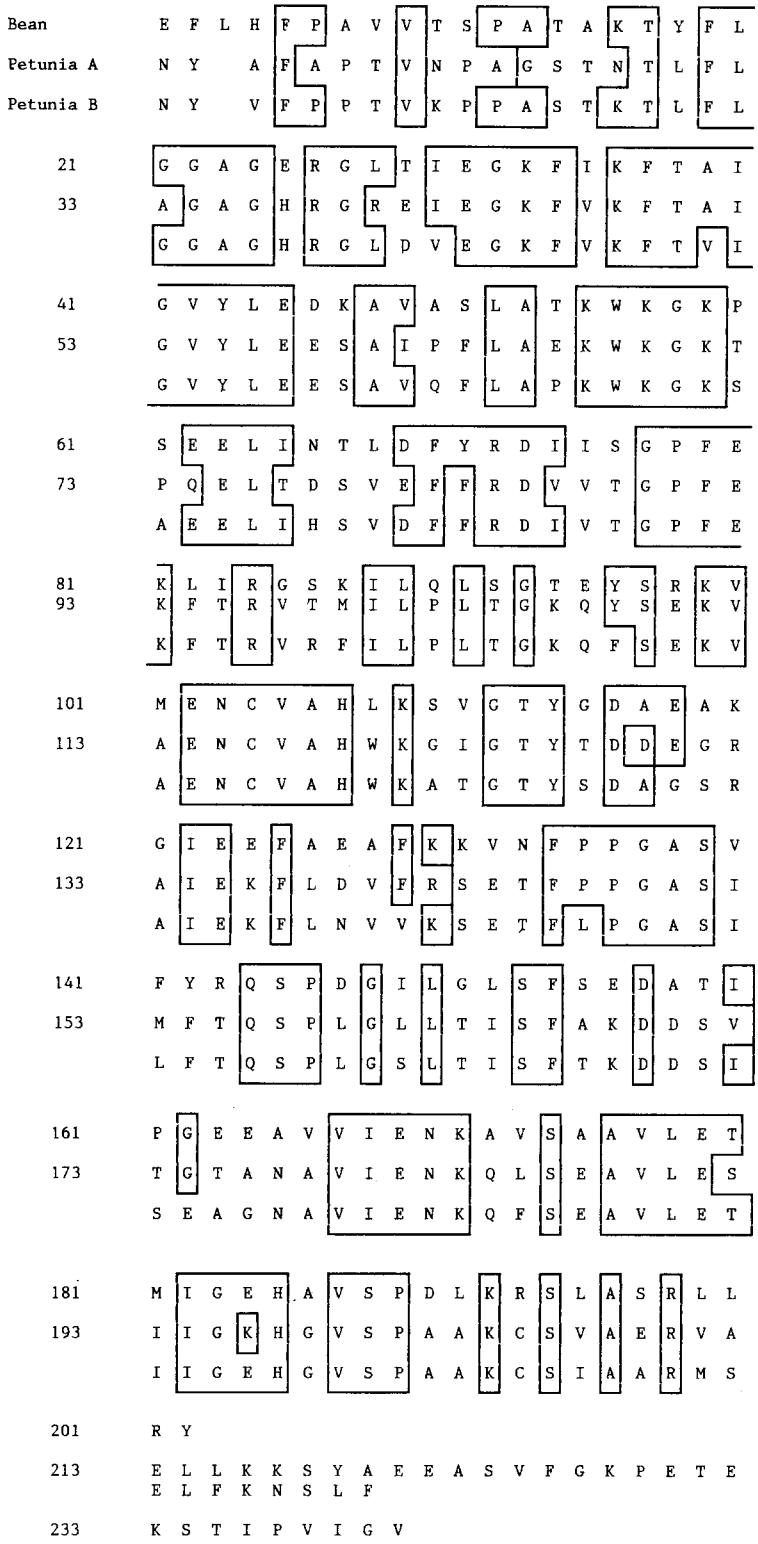

Fig. 2. Comparison of the derived amino acid sequences of chalcone isomerases from bean (CHI1) and *Petunia hybrida* (CHIA and CHIB).

Chalcone isomerase (CHI, E.C. 5.5.1.6) catalyzes the stereospecific isomerization of chalcones to their corresponding minus (–) flavanones, a key reaction in the biosynthesis of flavonoid pigments and, in the Leguminosae, in the formation of antimicrobial isoflavonoids [1]. A λgt 11 expression library was constructed from mRNA from bean (*Phaseolus vulgaris*) suspension cells treated with fungal elicitor [2]. This treatment results in increased levels of CHI protein and enzyme activity [3]. The library was screened with an antibody raised against purified CHI from the bean cultures [3], and two putative CHI clones were isolated [2]. The approximately 800 bp insert in clone pCHI1 was identified as encoding CHI based on the ability of the insert sequence to hybrid select an mRNA encoding a 27 kDa polypeptide recognized by the anti-(bean CHI) serum [2]. Use of this insert in northern blot experiments revealed induction of bean CHI transcripts in elicitor-treated cell suspension cultures, wounded hypocotyls, and hypocotyls infected with compatible and incompatible races of the fungal pathogen *Colletotrichum lindemuthianum* [2].

We now report the nucleotide and deduced amino acid sequence of the insert in clone pCHI1. The insert sequenced consisted of 808 nucleotides, comprising 185 nucleotides of 3′-untranslated region (with three potential polyadenylation signals), a short poly(A) tail, and 606 nucleotides of coding sequence (Fig. 1). Comparison with other CHI sequences [4] and with the M_r of the bean protein [3] suggests that the clone is lacking approximately 36 nucleotides of the 5′ coding sequence. pCHI1 was, however, the longest CHI clone obtained from the λgt 11 library. At the amino acid level, the bean CHI is 59% homologous to the CHIA and CHIB polypeptides from *Petunia hybrida* [4] (Fig. 2). The regions of homology occur in short blocks throughout the sequence. This might explain the lack of any strong antigenic cross-reactivity between the CHIs from bean and *Petunia* [5]. Hydropathy plots for the bean and *Petunia* enzymes are, however, extremely similar (data not shown).

CHI from bean catalyzes the isomerization of both 2′,4,4′-trihydroxychalcone and 2′,4,4′,6′-tetrahydroxychalcone to their corresponding flavanones, whereas the *Petunia* enzyme is inactive with the trihydroxychalcone [5]. These specificities reflect the A-ring substitution patterns of the flavonoid derivatives found in the two plant species [1]. The phytoalexins of *Petunia* are not isoflavonoids, so in this species CHI functions specifically in the formation of flavonoid pigments from 2′,4,4′,6′-tetrahydroxychalcone. The trihydroxychalcone A-ring substitution pattern is common to most of the antimicrobial isoflavonoids of the Leguminosae. Work is currently in progress, using molecular modeling and site-directed mutagenesis, to understand the molecular basis of the different substrate specificities of the CHIs from bean and *Petunia*.

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References

1. Dixon RA, Dey PM, Lamb CJ: Phytoalexins: enzymology and molecular biology. Adv Enzymol Relat Areas Mol Biol 55: 1–136 (1983).
2. Mehdy MC, Lamb CJ: Chalcone isomerase cDNA cloning and mRNA induction by fungal elicitor, wounding and infection. EMBO J 6: 1527–1533 (1987).
3. Robbins MP, Dixon RA: Induction of chalcone isomerase in elicitor-treated bean cells. Comparison of rates of synthesis and appearance of immunodetectable enzyme. Eur J Biochem 145: 195–202 (1984).
4. Van Tunen AJ, Hartman SA, Mur LA, Mol JNM: Regulation of chalcone flavanone isomerase (CHI) gene expression in *Petunia hybrida*: the use of alternative promoters in corolla, anthers and pollen. Plant Mol Biol 12: 539–551 (1989).
5. Dixon RA, Blyden ER, Robbins MP, van Tunen AJ, Mol JNM: Comparative biochemistry of chalcone isomerases. Phytochemistry 27: 2801–2808 (1988).