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Nucleotide sequence of a mutant acetolactate synthase gene from an imidazolinone-resistant *Arabidopsis thaliana* var. Columbia

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Acetolactate synthase (ALS) is the key enzyme in the biosynthesis of leucine, isoleucine, and valine. ALS is inhibited by three groups of herbicides: imidazolinones, sulfonylureas, and triazolo pyrimidines (2). To understand the molecular basis of imidazolinone herbicide resistance, a mutant GH-90 of *Arabidopsis thaliana*, isolated and characterized by Drs. George Haughn and Chris Somerville (1) was analyzed. The mutant plant and its ALS are 100 times more resistant to the imidazolinone herbicide imazapyr than the wild type. A genomic DNA library was constructed from the GH-90 DNA in the lambda vector EMBL3 and screened by an ALS gene probe. Among the three positively hybridizing clones identified, one was selected and purified. The ALS gene was subcloned in a 5.8 kb XbaI fragment into pUC18 and pBluescript KS+/−. Nested deletions were created using ExonucleaseIII and Mung bean nuclease treatment. Some regions were subcloned into pBluescript KS+/−. The DNA sequence was determined by both double and single stranded sequencing techniques using the dideoxy chain termination method. A point mutation from G to A was identified in the ALS gene at the 1958th nucleotide (underlined) of the coding sequence. This would result in Ser to Asn substitution at the 653rd amino acid (bold letter) of the matured ALS. This is a unique mutation in the ALS gene from the imazapyr-resistant *Arabidopsis* compared to the mutations conferring sulfonylurea resistance.

REFERENCES

1. Haughn,G.W. and Somerville,C. (1990) *Plant Physiology* in press.
2. Mazur,B.J. and Falco,S.C. (1989) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40**, 441−470.

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0001  CTTGTATCCATTCTCTTAACCAATAAAAAAAGAAAGAAAGATCAATTTGATAAATTTCTCAGCCACAAATTCTACATTTAGGTTTTAGCATATCGAAGGCTCAATCACAA

0111  ATACAATAGATAGACTAGAGATTCCAGCGTCACGTGAGTTTTATCTATAAATAAAGGACCAAAAATCAAATCCCGAGGGCATTTTCGTAATCCAACATAAAACCCTTAAA

0221  CTTCAAGTCTCATTTTTAAACAAATCATGTTCACAAGTCTCTTCTTCTTCTCTGTTTCTCTATCTCTTGCTCATCTTTCTCCTGAACCATGGCGGCGGCAACAACAACAA
                                                                                                  M  A  A  A  T  T  T  T
0331  CAACAACATCTTCTTCGATCTCCTTCTCCACCAAACCATCTCCTTCCTCCTCCAAATCACCATTACCAATCTCCAGATTCTCCCTCCCATTCTCCCTAAACCCCAACAAA
        T  T  S  S  S  I  S  F  S  T  K  P  S  P  S  S  S  K  S  P  L  P  I  S  R  F  S  L  P  F  S  L  N  P  N  K
0441  TCATCCTCCTCCTCCCGCCGCCGCGGTATCAAATCCAGCTCTCCCTCCTCCATCTCCGCCGTGCTCAACACAACCACCAATGTCACAACCACTCCCTCTCCAACCAAACC
      S  S  S  S  S  R  R  R  G  I  K  S  S  S  P  S  S  I  S  A  V  L  N  T  T  T  N  V  T  T  T  P  S  P  T  K  P
0551  TACCAAACCCGAAACATTCATCTCCCGATTCGCTCCAGATCAACCCCGCAAAGGCGCTGATATCCTCGTCGAAGCTTTAGAACGTCAAGGCGTAGAAACCGTATTCGCTT
        T  K  P  E  T  F  I  S  R  F  A  P  D  Q  P  R  K  G  A  D  I  L  V  E  A  L  E  R  Q  G  V  E  T  V  F  A  Y
0661  ACCCTGGAGGTGCATCAATGGAGATTCACCAAGCCTTAACCCGCTCTTCCTCAATCCGTAACGTCCTTCCTCGTCACGAACAAGGAGGTGTATTCGCAGCAGAAGGATAC
        P  G  G  A  S  M  E  I  H  Q  A  L  T  R  S  S  S  I  R  N  V  L  P  R  H  E  Q  G  G  V  F  A  A  E  G  Y
0771  GCTCGATCCTCAGGTAAACCAGGTATCTGTATAGCCACTTCAGGTCCCGGAGCTACAAATCTCGTTAGCGGATTAGCCGATGCGTTGTTAGATAGTGTTCCTCTTGTAGC
      A  R  S  S  G  K  P  G  I  C  I  A  T  S  G  P  G  A  T  N  L  V  S  G  L  A  D  A  L  L  D  S  V  P  L  V  A
0881  AATCACAGGACAAGTCCCTCGTCGTATGATTGGTACAGATGCGTTTCAAGAGACTCCGATTGTTGAGGTAACGCGTTCGATTACGAAGCATAACTATCTTGTGATGGATG
        I  T  G  Q  V  P  R  R  M  I  G  T  D  A  F  Q  E  T  P  I  V  E  V  T  R  S  I  T  K  H  N  Y  L  V  M  D  V
0991  TTGAAGATATCCCTAGGATTATTGAGGAAGCTTTCTTTTTAGCTACTTCTGGTAGACCTGGACCTGTTTTGGTTGATGTTCCTAAAGATATTCAACAACAGCTTGCGATT
        E  D  I  P  R  I  I  E  E  A  F  F  L  A  T  S  G  R  P  G  P  V  L  V  D  V  P  K  D  I  Q  Q  Q  L  A  I
1101  CCTAATTGGGAACAGGCTATGAGATTACCTGGTTATATGTCTAGGATGCCTAAACCTCCGGAAGATTCTCATTTGGAGCAGATTGTTAGGTTGATTTCTGAGTCTAAGAA
      P  N  W  E  Q  A  M  R  L  P  G  Y  M  S  R  M  P  K  P  P  E  D  S  H  L  E  Q  I  V  R  L  I  S  E  S  K  K
1211  GCCTGTGTTGTATGTTGGTGGTGGTTGTTTGAATTCTAGCGATGAATTGGGTAGGTTTGTTGAGCTTACGGGGATCCCTGTTGCGAGTACGTTGATGGGGCTGGGATCTT
        P  V  L  Y  V  G  G  G  C  L  N  S  S  D  E  L  G  R  F  V  E  L  T  G  I  P  V  A  S  T  L  M  G  L  G  S  Y
1321  ATCCTTGTGATGATGAGTTGTCGTTACATATGCTTGGAATGCATGGGACTGTGTATGCAAATTACGCTGTGGAGCATAGTGATTTGTTGTTGGCGTTTGGGGTAAGGTTT
        P  C  D  D  E  L  S  L  H  M  L  G  M  H  G  T  V  Y  A  N  Y  A  V  E  H  S  D  L  L  L  A  F  G  V  R  F
1431  GATGATCGTGTCACGGGTAAGCTTGAGGCTTTTGCTAGTAGGGCTAAGATTGTTCATATTGATATTGACTCGGCTGAGATTGGGAAGAATAAGACTCCTCATGTGTCTGT
      D  D  R  V  T  G  K  L  E  A  F  A  S  R  A  K  I  V  H  I  D  I  D  S  A  E  I  G  K  N  K  T  P  H  V  S  V
1541  GTGTGGTGATGTTAAGCTGGCTTTGCAAGGGATGAATAAGGTTCTTGAGAACCGAGCGGAGGAGCTTAAGCTTGATTTTGGAGTTTGGAGGAATGAGTTGAACGTACAGA
        C  G  D  V  K  L  A  L  Q  G  M  N  K  V  L  E  N  R  A  E  E  L  K  L  D  F  G  V  W  R  N  E  L  N  V  Q  K
1651  AACAGAAGTTTCCGTTGAGCTTTAAGACGTTTGGGGAAGCTATTCCTCCACAGTATGCGATTAAGGTCCTTGATGAGTTGACTGATGGAAAAGCCATAATAAGTACTGGT
        Q  K  F  P  L  S  F  K  T  F  G  E  A  I  P  P  Q  Y  A  I  K  V  L  D  E  L  T  D  G  K  A  I  I  S  T  G
1761  GTCGGGCAACATCAAATGTGGGCGGCGCAGTTCTACAATTACAAGAAACCAAGGCAGTGGCTATCATCAGGAGGCCTTGGAGCTATGGGATTTGGACTTCCTGCTGCGAT
      V  G  Q  H  Q  M  W  A  A  Q  F  Y  N  Y  K  K  P  R  Q  W  L  S  S  G  G  L  G  A  M  G  F  G  L  P  A  A  I
1871  TGGAGCGTCTGTTGCTAACCCTGATGCGATAGTTGTGGATATTGACGGAGATGGAAGCTTTATAATGAATGTGCAAGAGCTAGCCACTATTCGTGTAGAGAATCTTCCAG
        G  A  S  V  A  N  P  D  A  I  V  V  D  I  D  G  D  G  S  F  I  M  N  V  Q  E  L  A  T  I  R  V  E  N  L  P  V
1981  TGAAGGTACTTTTATTAAACAACCAGCATCTTGGCATGGTTATGCAATGGGAAGATCGGTTCTACAAAGCTAACCGAGCTCACACATTTCTCGGGGATCCGGCTCAGGAG
        K  V  L  L  L  N  N  Q  H  L  G  M  V  M  Q  W  E  D  R  F  Y  K  A  N  R  A  H  T  F  L  G  D  P  A  Q  E
2091  GACGAGATATTCCCGAACATGTTGCTGTTTGCAGCAGCTTGCGGGATTCCAGCGGCGAGGGTGACAAAGAAAGCAGATCTCCGAGAAGCTATTCAGACAATGCTGGATAC
      D  E  I  F  P  N  M  L  L  F  A  A  A  C  G  I  P  A  A  R  V  T  K  K  A  D  L  R  E  A  I  Q  T  M  L  D  T
2201  ACCAGGACCTTACCTGTTGGATGTGATTTGTCCGCACCAAGAACATGTGTTGCCGATGATCCCGAATGGTGGCACTTTCAACGATGTCATAACGGAAGGAGATGGCCGGA
        P  G  P  Y  L  L  D  V  I  C  P  H  Q  E  H  V  L  P  M  I  P  N  G  G  T  F  N  D  V  I  T  E  G  D  G  R  I
2311  TTAAATACTGAGAGATGAAACCGGTGATTATCAGAACCTTTTATGGTCTTTGTAT
        K  Y  Stop
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