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(54) **SUPEROXIDE DISMUTASE (SOD) GENE AND A METHOD OF IDENTIFYING AND CLONING THEREOF**

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C12N	***15/00***	(2006.01)

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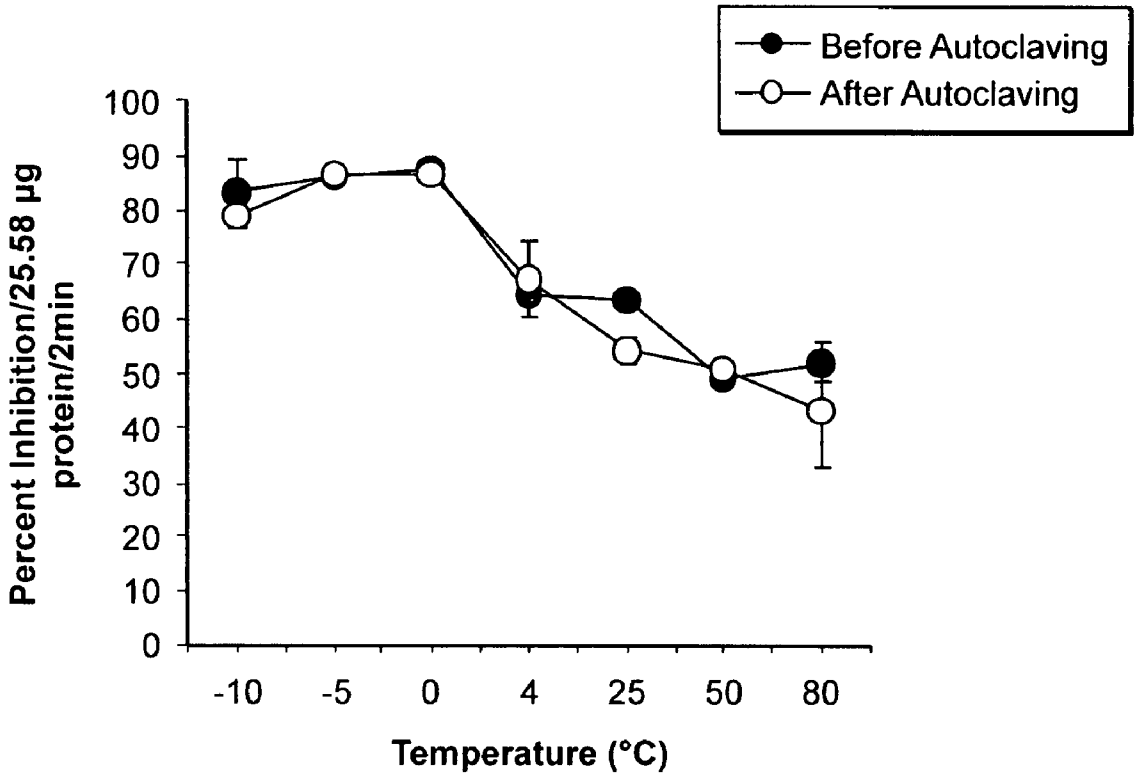
(57) **ABSTRACT**

The present invention provides a superoxide dismutase gene from *Potentilla atrosanguinea,* a construct containing the gene coding for superoxide dismutase and transformed *E.coli* producing the SOD protein.



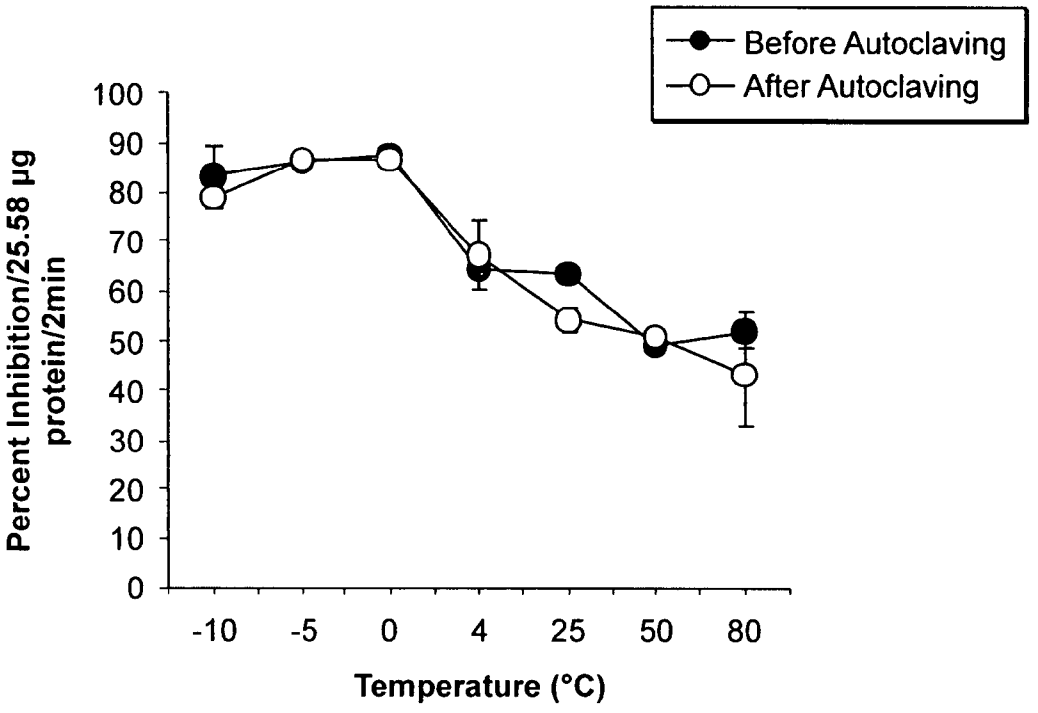


FIG. 1

FIG. 2A
FIG. 2B
FIG. 2C

FIG. 2

Comparison of the nucleotide sequence of the *Potentilla* Cu/Zn SOD with sequences from other plant species. Regions of complete homology are indicated with asterisks.

```
Malus          ATGGTGAAGGGTGTTGCTGTTCTCGGCTCCAGTGAGGGCGTTAAAGGAACCATCAGCTTT
Potentilla     ATGGCAAAGGGCGTTGCTGTACTTAGCTCCAGTGAGGGTGTTGCTGGAACTATCCTCTTT
Populus        ATGGTGAAGGCTGTAGCTGTTCTTAATAGCAGTGAAGGTGTGAGTGGCACCATCTTCTTT
Pea            ATGGTGAAGGCTGTGGCAGTTCTTAGTAACAGTAACGAAGTCTCGGGTACTATTAACTTC
Arabidopsis    ATGGCGAAAGGAGTTGCAGTTTTGAACAGCAGTGAGGGTGTTACGGGGACTATCTTTTTC
Oryza          ATGGTGAAGGCTGTTGCTGTGCTTGCTAGCAGTGAGGGTGTCAAGGGCACCATCTTTTTC
               ****  ** *  ** ** **  *     **** * *  **    ** ** **    **

Malus          GTCCAGGAGGGAGATGGCCCAACTACTGTGACTGGAAGTGTCTCTGGCCTCAAGCCTGGA
Potentilla     ACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGCCTGGG
Populus        ACCCAAGAAGGAGATGGCCCAACTACTGTAATTGGAAACCTTTCTGGTCTTAAGCCAGGC
Pea            AGTCAGGAGGGAAATGGTCCAACCACTGTAACTGGAACTCTTGCTGGTCTTAAGCCTGGC
Arabidopsis    ACCCAGGAAGGCGATGGTGTGACCACTGTGAGTGGAACAGTTTCTGGCCTTAAGCCTGGT
Oryza          TCCCAAGAGGGAGATGGTCCGACCTCTGTGACGGGAAGTGTCTCTGGGCTCAAGCCAGGG
                  ** ** **  ****    **  **** *  ****   *  **** ** ***** **
```

FIG. 2A

```
Malus          CTTCATGGTTTCCATGTCCATGCTCTTGGAGACACAACAAACGGTTGCATGTCAACTGGG
Potentilla     CTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAACTGGA
Populus        CTTCATGGCTTCCACGTCCATGCCCTTGGAGACACCACAAATGGCTGCATGTCAACTGGG
Pea            CTCCACGGCTTCCATATCCATGCCTTGGGAGACACCACAAACGGTTGCATTTCAACTGGA
Arabidopsis    CTTCATGGTTTCCATGTCCATGCTCTTGGTGACACCACTAACGGTTGCATGTCTACTGGT
Oryza          CTCCATGGATTCCATGTGCACGCGCTCGGTGACACCACTAATGGCTGCATGTCAACTGGA
               ** ** ** *****  * ** **  * ** ***** ** ** ** ***** ** *****

Malus          CCACACTTCAATCCTGCTGGAAAAGAGCATGGTGCCCCTGAAGATGAGCTTCGCCATGCT
Potentilla     CCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCATGCT
Populus        CCGCATTTTAATCCTGTAGGCAAGGAGCATGGTGCCCCTGAGGATGAGAATCGTCATGCT
Pea            CCACATTTCAATCCTAATGGGAAGGAACATGGTGCCCCTGAGGATGAGACTAGACATGCT
Arabidopsis    CCACATTTCAACCCCGATGGTAAAACACACGGTGCCCCTGAGGATGCTAATCGACATGCT
Oryza          CCACACTTCAATCCTACTGGGAAGGAACATGGGGCACCACAAGATGAGAACCGCCATGCC
               ** ** ** ** **    ** **    ** **  * **  * ****      * *****

Malus          GGCGATCTTGGAAACATCACTGCTGGGGACGATGGAACTGCAACCTTCACGATTGTTGAC
Potentilla     GGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTGTTGAC
Populus        GGTGATCTGGGAAATGTCACTGTTGGTGATGATGGCACTGCTGCTTTCACAATCATTGAC
Pea            GGTGATCTTGGAAATATCAATGTTGGTGATGATGGAACTGTAAGCTTCACCATTACTGAC
Arabidopsis    GGTGATCTAGGAAACATCACTGTTGGAGATGATGGAACTGCCACCTTCACAATCACTGAT
Oryza          GGTGATCTTGGAAATATAACAGCTGGAGCAGATGGTGTTGCTAATGTCAATGTCTCTGAC
               ** ***** *****  * *  * *** *  ** **   **      ***   *   ***
```

FIG. 2B

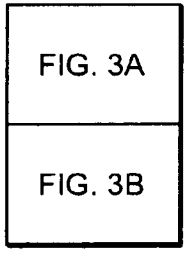
```
Malus          AAGCAGATTCCTCTCGCTGGACCACACTCTATCATTGGTAGGGCGGTTGTTGTCCACGCA
Potentilla     AAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCCATGCA
Populus        AAACAGATTCCTCTTACTGGACCACATTCCATTATTGGTTGGGCTGTTGTTGTTCATGGA
Pea            AACCATATCCCTCTCACTGGAACAAACTCCATCATAGGAAGGGCTGTTGTTGTCCATGCC
Arabidopsis    TGCCAGATTCCTCTTACTGGACCAAACTCTATTGTTGGTAGGGCTGTTGTTGTCCATGCA
Oryza          AGCCAGATCCCCCTTACTGGAGCACACTCCATCATTGGCCGAGCTGTTGTTGTCCATGCT
                  ** ** ** **  ***** ** * ** **  * **  * ** ******** ** *

Malus          GACCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACAGGAAATGCTGGT
Potentilla     GATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGAAATGCTGGT
Populus        GATCCTGATGATCTTGGCAAGGGAGGACATGAACTCAGCAAAACCACTGGTAATGCTGGC
Pea            GATCCTGATGATCTTGGGAAAGGTGGTCACGAGCTTAGCAAAACTACTGGAAATGCTGGT
Arabidopsis    GACCCTGATGACCTCGGAAAGGGAGGCCATGAACTCAGCCTGGCTACTGGAAACGCAGGC
Oryza          GATCCTGATGATCTTGGCAAGGGTGGACATGAGCTTAGCAAGACCACTGGAAATGCTGGG
               ** ******** ** ** ** ** ** ** ** ** ***    * ** ** ** ** **

Malus          GGCAGGGTGGCTTGCGGTATTATTGGTCTGCAAGGATGA
Potentilla     GGCAGGATAGCTTGTGGTATTATTGGCCTTCAAGGATGA
Populus        GGCAGAGTAGCATGCGGTATTATTGGTCTGCAAGGTTGA
Pea            GGCAGAGTAGCTTGTGGTATTATTGGGTTGCAAGGATAG
Arabidopsis    GGCCGTGTTGCTTGCGGCATCATTGGTCTCCAGGGCTAA
Oryza          GGCCGAGTTGCTTGCGGAATCATCGGACTCCAGGGTTAG
```

FIG. 2C

FIG. 3



Comparison of the deduced amino acid sequence of the *Potentilla* Cu/Zn SOD with sequences from other plant species. Regions of complete homology are indicated with asterisks.

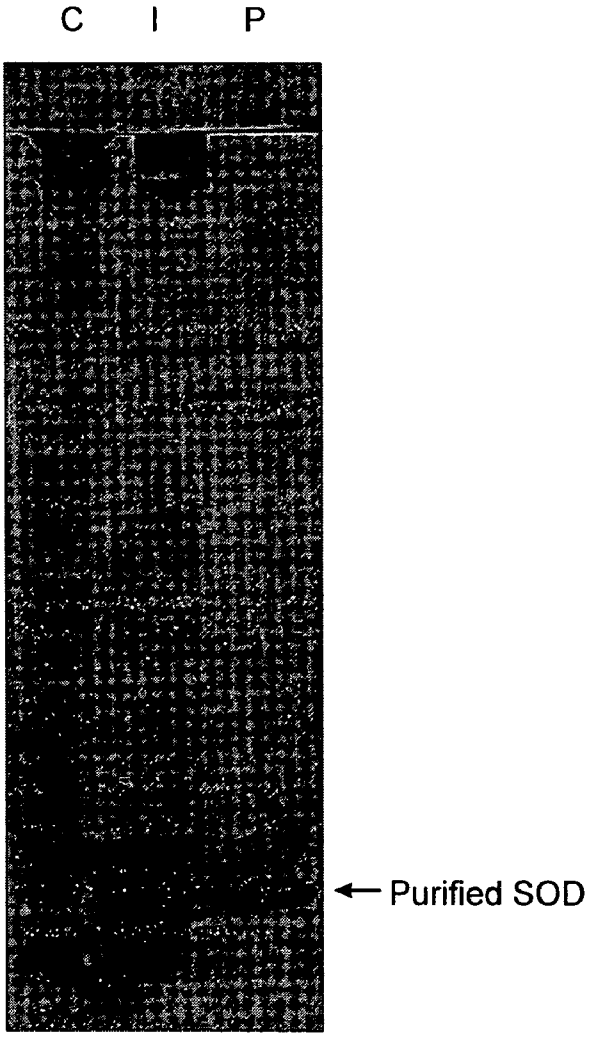
```
Malus          MVKGVAVLGSSEGVKGTISFVQEGDGPTTVTGSVSGLKPGLHGFHVHALGDTTNGCMSTG
Potentilla     MAKGVAVLSSSEGVAGTILFTQEGDGPTTVTGNISGLKPGLHGFHVHALGDTTNGCMSTG
Arabidopsis    MAKGVAVLNSSEGVTGTIFFTQEGDGVTTVSGTVSGLKPGLHGFHVHALGDTTNGCMSTG
Populus        MVKAVAVLNSSEGVSGTIFFTQEGDGPTTVTGNLSGLKPGLHGFHVHALGDTTNGCMSTG
Oryza          MVKAVVVLGSSEIVKGTIHFVQEGDGPTTVTGSVSGLKPGLHGFHIHALGDTTNGCMSTG
Zea            MVKAVAVLGSSEGVKGTIFFTQEGDGPTTVTGSVSGLKPGLHGFHVHALGDTTNGCMSTG
Gossypium      MVKAVAVLGSNEGVSGTVFFSQEGDGPTTVTGNLSGLKPGLHGFHVHALGDTTNGCMSTG
Pisum          MVKAVAVLSNSNEVSGTINFSQEGNGPTTVTGTLAGLKPGLHGFHIHALGDTTNGCISTG
Soybean        MVKAVAVLGSSEGVTGTIFFTQEGNGPTTVTGSLAGLKPGLHGFHVHALGDTTNGCLSTG
               *.*.*.**...: * **: * ***:* ***:*.::**********:**********:***
```

FIG. 3A

```
Malus          PHFNPAGKEHGAPEDELRHAGDLGNITAGDDGTATFTIVDKQIPLAGPHSIIGRAVVVHA
Potentilla     PHFNPAGKEHGSPEDETRHAGDLGNITVGDDGTACFTIVDKQIPLTGPHSIIGRAVVVHA
Arabidopsis    PHFNPDGKTHGAPEDANRHAGDLGNITVGDDGTATFTITDCQIPLTGPNSIVGRAVVVHA
Populus        PHFNPVGKEHGAPEDENRHAGDLGNVTVGDDGTAAFTIIDFQIPLTGPHSIIGRAVVVHG
Oryza          PHYNPAGKEHGAPEDETRHAGDLGNVTAGEDGVANIHVVDSQIPLTGPNSIIGRAVVVHA
Zea            PHYNPASKEHGAPEDENRHAGDLGNVTAGADGVANINVTDSQIPLTGPNSIIGRAVVVHA
Gossypium      PHFNPAGKEHGAPEDENRHAGDLGNVTVGDDGCASFSITDKQIPLTGPNSIIGRAVVVHA
Pisum          PHFNPNGKEHGAPEDETRHAGDLGNINVGDDGTVSFTITDNHIPLTGTNSIIGRAVVVHA
Soybean        AHFNPNNNEHGAPEDENRHAGDLGNVNVGDDGTVSFSITDSQIPLTGPNSIIGRAVVVHA
               .*:** .: **:***  ********:..* ** . : : * :***:*.:**:*******.

Malus          DPDDLGKGGHELSKSTGNAGGRVACGIIGLQG
Potentilla     DPDDLGKGGHELSKSTGNAGGRIACGIIGLQG
Arabidopsis    DPDDLGKGGHELSLATGNAGGRVACGIIGLQG
Populus        DPDDLGKGGHELSKTTGNAGGRVACGIIGLQG
Oryza          DPDDLGKGGHELSKTTGNAGGRVACGIIGLQG
Zea            DPDDLGKGGHELSKSTGNAGGRVACGIIGLQG
Gossypium      DPDDLGKGGHELSKSTGNAGGRVACGIIGLQG
Pisum          DPDDLGKGGHELSKTTGNAGGRVACGIIGLQG
Soybean        DSDDLGKGGHELSKTTGNAGGRVACGIIGLQG
               *.*********** :*******:*********
```

FIG. 3B



Expression and purification of *Potentilla* SOD in *E. coli.*

C, Control;
I, Protein induced by IPTG;
P, Purified SOD.

The gel was stained by silver staining.

FIG. 4A

Activity staining of the gel to depict the activity of purified SOD.

P, Purified SOD.

FIG. 4B

FIG. 5

result of alignment of present sod gene with the sod gene of other plant species

Sequence 1: gi|311970|gi|311970I.batatas mRNA for superoxide dismutase
Length = 459 (1 .. 459)

Sequence 2: lcl|IHBT-potentilla
Length = 459 (1 .. 459)

FIG. 5-1

```
Score =  348 bits (181),  Expect = 8e-93
 Identities = 323/394 (81%), Gaps = 0/394 (0%)
 Strand=Plus/Plus

Query  56   TCTTCAGCCAAGAAGGAGATGGTCCAACCACAGTCACTGGAAACGTTTCGGGCCTCAAAC  115
            |||| | |||||| |||||||| ||||| || || || |||||| |||| |||||||| |
Sbjct  56   TCTTTACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGC  115

Query  116  CTGGTCTTCATGGCTTCCATGTCCATGCCCTAGGTGACACAACAAATGGATGCATGTCTA  175
            |||| |||||||| |||||||| ||||| || || |||||||| ||||| |||||||| |
Sbjct  116  CTGGGCTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAA  175

Query  176  CTGGACCACATTTCAATCCTGCTGGAAAGGAGCATGGAGCTCCTGGAGACGATAACCGCC  235
            |||||||||||||||||||||||| || |||||||  ||||||  ||| || |  || |
Sbjct  176  CTGGACCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTC  235

Query  236  ATGCCGGTGATCTTGGAAACATCACGGTTGGAGAAGATGGTACTGCTTCATTCACCATCA  295
            |||| |||||||||||||| ||||| ||||| || || || |||||||  ||||| ||
Sbjct  236  ATGCTGGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTG  295

Query  296  CTGACAAGCAGATTCCGCTTACTGGAGCAAATTCTGTTATTGGAAGAGCTGTTGTTGTTC  355
             ||||||  |||||||  || ||||||  || | |||  | |||||  || ||||||||||| |
Sbjct  296  TTGACAAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCC  355
```

FIG. 5-2

```
Query  356  ATGGTGATCCCGATGATCTTGGTAAAGGTGGCCATGAGCTCAGCAAAAGCACTGGAAATG  415
            |||  ||||| ||||| ||||| || ||||| |||||||| ||||||  ||||||||||
Sbjct  356  ATGCAGATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGAAATG  415

Query  416  CTGGCGGGAGGGTTGCCTGCGGTATCATTGGCCT  449
            |||| || ||| | || || ||||| ||||||||
Sbjct  416  CTGGTGGCAGGATAGCTTGTGGTATTATTGGCCT  449
```

□

> gi|38228696|emb|AJ586519.1| Fagus sylvatica partial sod1 mRNA for superoxide dismutase
> Length=710

Score = 379 bits (191), Expect = 5e-102
Identities = 392/459 (85%), Gaps = 0/459 (0%)
Strand=Plus/Plus

```
Query  1    ATGGCAAAGGGCGTTGCTGTACTTAGCTCCAGTGAGGGTGTTGCTGGAACTATCCTCTTT  60
            ||||| ||||| || ||||| |||||||| | ||||||||||  ||| ||||||  ||||
Sbjct  43   ATGGCCAAGGGTGTGGCTGTTCTTAGCTCGAATGAGGGTGTTTGTGGCACTATCTACTTT  102

Query  61   ACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGCCTGGG  120
             ||||||| |||||||||||||||||| || || ||||| |||||||||| || |||||
Sbjct  103  GCCCAAGAAGGAGATGGCCCAACTACAGTAACTGGAAATATTTCTGGCCTTAAACCTGGA  162
```

FIG. 5-3

```
Query  121  CTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAACTGGA  180
            ||  |||||  |||||  ||  ||||||||||||||||||  ||||||||||||||||||||
Sbjct  163  CTCCATGGCTTCCACGTGCATGCTCTTGGGGACACAACAAATGGTTGCATGTCAACTGGA  222

Query  181  CCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCATGCT  240
            ||||||||||||||||||||||||||||||  |||||||  ||||  ||  ||||||||||
Sbjct  223  CCACATTTCAATCCTGCTGGCAAAGAGCATGGTGCTCCTGAGGATGCGAATCGTCATGCT  282

Query  241  GGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTGTTGAC  300
            |||||||  ||||||  |||  ||||||  |||||  ||  ||  |   |  ||||||||  |||||
Sbjct  283  GGTGATCTGGGAAATGTCAATGTTGGTGATGATGGCACAGTCAGTTTCACAATAATTGAC  342

Query  301  AAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCCATGCA  360
            ||||||||||  ||   |||  |||  |  ||  ||  ||  ||  ||||||||||||||||||  |
Sbjct  343  AAACAGATTCCACTTTGTGGTCCAAATTCCATTATCGGAAGGGCTGTTGTTGTCCATGGA  402

Query  361  GATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGAAATGCTGGT  420
            |||||  |||||  ||||||||||  ||||||||  ||||||||   ||||||||||||||||
Sbjct  403  GATCCAGATGATCTTGGCAAGGGGGGGACATGAACTTAGCAAGAGCACTGGAAATGCTGGT  462

Query  421  GGCAGGATAGCTTGTGGTATTATTGGCCTTCAAGGATGA  459
            |||  |  ||||||||||||||  |||||  ||  |||||||||
Sbjct  463  GGCCGTATAGCTTGTGGTATCATTGGTCTCCAAGGATGA  501
```

FIG. 5-4

□

> gi|4102858|gb|AF016892.1|AF016892 Populus tremuloides cytoplasmic superoxide dismutase 1 (SODcyt1) mRNA, complete cds
Length=787

Score = 333 bits (168), Expect = 3e-88
Identities = 342/400 (85%), Gaps = 0/400 (0%)
Strand=Plus/Plus

```
Query  56   TCTTTACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGC  115
            ||||||||||||| ||||||||||||||||||| |  |||||| ||||||| || ||||
Sbjct  134  TCTTTACCCAAGAAGGAGATGGCCCAACTACTGTAATTGGAAACCTTTCTGGTCTTAAGC  193

Query  116  CTGGGCTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAA  175
            | || |||||||| ||||| || ||||| ||||| ||||| || ||||| ||||||||||
Sbjct  194  CAGGCCTTCATGGCTTCCACGTCCATGCCCTTGGAGACACCACAAATGGCTGCATGTCAA  253

Query  176  CTGGACCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTC  235
            ||||  ||  ||||| |||||||  ||||| ||||||||  | ||||| ||||||| |||||
Sbjct  254  CTGGGCCGCATTTTAATCCTGTAGGCAAGGAGCATGGTGCCCCTGAGGATGAGAATCGTC  313
```

FIG. 5-5

```
Query  236  ATGCTGGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTG  295
            ||||||||||||| |||||| |||||||||| ||||| || ||||||   ||||||||
Sbjct  314  ATGCTGGTGATCTGGGAAATGTCACTGTTGGTGATGATGGCACTGCTGCTTTCACAATCA  373

Query  296  TTGACAAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCC  355
            |||||||||||||||||| |||||||||| || || |||||| ||||||||||||| |
Sbjct  374  TTGACAAACAGATTCCTCTTACTGGACCACATTCCATTATTGGTTGGGCTGTTGTTGTTC  433

Query  356  ATGCAGATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGAAATG  415
            ||| |||||||||||| |||||||||| |||||||| || |||||| ||||||| ||||
Sbjct  434  ATGGAGATCCTGATGATCTTGGCAAGGGAGGACATGAACTCAGCAAAACCACTGGTAATG  493

Query  416  CTGGTGGCAGGATAGCTTGTGGTATTATTGGCCTTCAAGG  455
            |||| |||||  |||| || |||||||||| || |||||
Sbjct  494  CTGGCGGCAGAGTAGCATGCGGTATTATTGGTCTGCAAGG  533
```

> gi|50540928|gb|AY642137.1| Manihot esculenta copper/zinc superoxide dismutase mRNA, complete cds

FIG. 5-6

```
Length=774

 Score =  333 bits (168),  Expect = 3e-88
 Identities = 354/416 (85%), Gaps = 0/416 (0%)
 Strand=Plus/Plus

Query  31   AGTGAGGGTGTTGCTGGAACTATCCTCTTTACCCAAGAGGGAGATGGCCCAACTACTGTG  90
            ||||||||||||||||| || ||| |||| |||||||| |||||||| ||||| || ||
Sbjct  44   AGTGAGGGTGTTGCTGGGACAATCTTCTTCACCCAAGAAGGAGATGGTCCAACCACCGTC  103

Query  91   ACCGGAAACATTTCTGGCCTCAAGCCTGGGCTTCATGGTTTCCATGTTCATGCTCTTGGG  150
            || ||||   |||||||||| ||||| |||||||||| |||||||||||| |||||
Sbjct  104  ACTGGAAGTGTTTCTGGCCTTAAGCCAGGGCTTCATGGATTCCATGTTCATGCCCTTGGA  163

Query  151  GACACAACCAATGGTTGCATGTCAACTGGACCACATTTCAATCCTGCTGGCAAAGAGCAT  210
            |||||||| |||||||||||||||||| |||||||||| |||| ||||||||||||
Sbjct  164  GACACAACAAATGGTTGCATGTCAACTGGGCCACATTTCAACCCTGGTGGCAAAGAGCAT  223

Query  211  GGGTCTCCTGAAGATGAGACTCGTCATGCTGGTGATCTTGGAAATATCACTGTTGGGGAT  270
            ||  | ||||| || || | |||||||||||||||| |||||| |||||| ||| |||
Sbjct  224  GGTGCCCCTGAGGACGACATTCGTCATGCTGGTGATCTGGGAAATGTCACTGCTGGTGAT  283
```

FIG. 5-7

```
Query  271  GACGGAACTGCTTGCTTCACAATTGTTGACAAACAGATTCCTCTCACTGGACCACACTCT  330
            || || |||||| | |||||||| |||||||  | ||||||||  |||| || || ||
Sbjct  284  GATGGCACTGCTAGTTTCACAATCGTTGACAAGGATATTCCTCTTTCTGGTCCGCATTCC  343

Query  331  ATCATTGGTAGGGCTGTTGTTGTCCATGCAGATCCTGATGACCTTGGCAAGGGTGGACAT  390
            ||  | || ||||| || ||||| ||||||||||||||||| ||||| ||||| ||||||
Sbjct  344  ATTGTAGGAAGGGCAGTCGTTGTTCATGCAGATCCTGATGATCTTGGAAAGGGGGGACAT  403

Query  391  GAGCTTAGCAAATCCACTGGAAATGCTGGTGGCAGGATAGCTTGTGGTATTATTGG  446
            || ||||||||| |||||||||||||||||||||| |||| |||||| |||||||
Sbjct  404  GAACTTAGCAAAACCACTGGAAATGCTGGTGGCAGGGTAGCATGTGGTGTTATTGG  459
```

□

> gi|6723475|emb|AJ279694.1|BPE279694 Betula pendula partial mRNA for copper/zinc-superoxide dismutase
> (cu/Zn sod gene)
> Length=355

Score = 323 bits (163), Expect = 3e-85
Identities = 289/331 (87%), Gaps = 0/331 (0%)
Strand=Plus/Plus

FIG. 5-8

```
Query  49   ACTATCCTCTTTACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGC  108
            ||||||| |||||||||||| |  ||||||||||||||| || || ||||| |||||||||
Sbjct  25   ACTATCCACTTTACCCAAGAAGCTGATGGCCCAACTACAGTAACTGGAAATATTTCTGGC  84

Query  109  CTCAAGCCTGGGCTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGC  168
             || |||||||||| ||||| |||||||| ||||| |||||||||||||| |||||||||
Sbjct  85   CTTAAGCCTGGGCTCCATGGGTTCCATGTCCATGCACTTGGGGACACAACAAATGGTTGC  144

Query  169  ATGTCAACTGGACCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAG  228
             |||||||||| ||||||||||||||||||||||||||||  ||||||| ||||||
Sbjct  145  ATGTCAACTGGGCCACATTTCAATCCTGCTGGCAAAGAGCATGGTGCTCCTGAGGATGAG  204

Query  229  ACTCGTCATGCTGGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTC  288
             | ||||||||| ||||||| |||||| |||| ||||| ||||| || |||||  | |||
Sbjct  205  AATCGTCATGCCGGTGATCTGGGAAATGTCACCGTTGGTGATGATGGTACTGCCAGTTTC  264

Query  289  ACAATTGTTGACAAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTT  348
             ||||| |||||||| |||||||| ||  |||||||||| ||||| ||||| |||||||||
Sbjct  265  ACAATAGTTGACAAGCAGATTCCACTTTCTGGACCACATTCTATTATTGGAAGGGCTGTT  324

Query  349  GTTGTCCATGCAGATCCTGATGACCTTGGCA  379
             |||||||| |  ||||| ||||| |||||||
Sbjct  325  GTTGTCCACGGGGATCCAGATGATCTTGGCA  355
```

FIG. 5-9

□

> gi|13274149|emb|AJ278669.1|PTR278669 [U] Populus tremula x Populus tremuloides mRNA for putative cytosolic CuZn-superoxide dismutase (cyt-SOD1 gene)
Length=851

Score = 305 bits (154), Expect = 6e-80
Identities = 331/390 (84%), Gaps = 0/390 (0%)
Strand=Plus/Plus

```
Query  57   CTTTACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGCC  116
            ||||||||||||| |||||||| |||||||||| || |||| | | | ||| || |||||
Sbjct  135  CTTTACCCAAGAAGGAGATGGTCCAACTACTGTAACTGGAAGCCTCTGTGGTCTTAAGCC  194

Query  117  TGGGCTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAAC  176
             || |||||||| |||||||||||||| ||||| ||||| || ||||| ||||||||||
Sbjct  195  AGGCCTTCATGGCTTCCATGTTCATGCCCTTGGAGACACCACAAATGGCTGCATGTCAAC  254

Query  177  TGGACCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCA  236
             ||| || ||||| ||||||| |||||||||||||| | ||||| ||||||| ||||||
Sbjct  255  TGGCCCGCATTTTAATCCTGTAGGCAAAGAGCATGGTGCCCCTGAGGATGAGAATCGTCA  314
```

FIG. 5-10

```
Query  237  TGCTGGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTGT  296
            |||||||||| | |||||| |||||||||| ||||| || || |||    || ||||  |
Sbjct  315  TGCTGGTGATTTGGGAAATGTCACTGTTGGTGATGATGGCACCGCTACTGTCTCAATCAT  374

Query  297  TGACAAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCCA  356
            |||||| |||||||||||||||||||| | || ||| |||| |||||||||||||| ||
Sbjct  375  TGACAACCAGATTCCTCTCACTGGACCAAATTCCATCGTTGGAAGGGCTGTTGTTGTTCA  434

Query  357  TGCAGATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGAAATGC  416
            |||||||||||||| |||||||||| ||||||| |||||||| ||||||  |||||
Sbjct  435  TGCAGATCCTGATGATCTTGGCAAGGGAGGACATGAACTTAGCAAAAGCACTGGTAATGC  494

Query  417  TGGTGGCAGGATAGCTTGTGGTATTATTGG  446
            |||||||||  |||| |||||| |||||||
Sbjct  495  TGGTGGCAGAGTAGCATGTGGTGTTATTGG  524
```

□

> gi|53748478|emb|AJ844003.1| Plantago major mRNA for copper-zinc superoxide dismutase (csd1 gene)
Length=779

Score = 305 bits (154), Expect = 6e-80
Identities = 373/446 (83%), Gaps = 0/446 (0%)
Strand=Plus/Plus

FIG. 5-11

```
Query  7    AAGGGCGTTGCTGTACTTAGCTCCAGTGAGGGTGTTGCTGGAACTATCCTCTTTACCCAA  66
            ||||| ||||| || ||||||  ||||||||||||  ||| ||  |||||||| |||||
Sbjct  70   AAGGGTGTTGCAGTGCTTAGCAGCAGTGAGGGTGTTAGTGGCACCGTCCTCTTTTCCCAA  129

Query  67   GAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGCCTGGGCTTCAT  126
            || ||||| || || || ||||| || |||||| |||||||||| |||||||| |||||
Sbjct  130  GAAGGAGAAGGACCCACCACTGTAACTGGAAACCTTTCTGGCCTTAAGCCTGGACTTCAC  189

Query  127  GGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAACTGGACCACAT  186
            || ||||||||||||||||||| ||||| ||||| |||||||||||| |||||||||
Sbjct  190  GGCTTCCATGTTCATGCTCTTGGTGACACTACCAACGGTTGCATGTCAACAGGACCACAT  249

Query  187  TTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCATGCTGGTGAT  246
            |||||||| |||| |||||||||| ||||||| |||||| ||| ||||||||||
Sbjct  250  TTCAATCCGGCTGCAAAAGAGCATGGTGCTCCTGATGATGAGGTTCGCCATGCTGGTGAC  309

Query  247  CTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTGTTGACAAACAG  306
            ||||| ||| |||| || || ||||| ||||||||  | ||||| |||||||||| | |
Sbjct  310  CTTGGTAATGTCACAGTGGGAGATGATGGAACTGCAAGTTTCACCATTGTTGACAAGCTG  369

Query  307  ATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCCATGCAGATCCT  366
            ||||| || |||||||||| || |||||||| ||||||||||||||||||| || ||
Sbjct  370  ATTCCGCTGACTGGACCACATTCCATCATTGGAAGGGCTGTTGTTGTCCATGCTGACCCC  429
```

FIG. 5-12

```
Query  367  GATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGAAATGCTGGTGGCAGG  426
            |||||  | || | ||||||||||| || |||||| | || ||||||||||||| ||
Sbjct  430  GATGATTTGGGAAGGGGTGGACATGAACTCAGCAAAACTACCGGAAATGCTGGTGGAAGA  489

Query  427  ATAGCTTGTGGTATTATTGGCCTTCA  452
             | |||||||||| ||||| |||||
Sbjct  490  GTTGCTTGTGGTATCATTGGTCTTCA  515
```

□

> gi|5726591|gb|AF170297.1|AF170297 Manihot esculenta copper/zinc-superoxide dismutase mRNA, complete cds
Length=801

```
 Score =  295 bits (149),  Expect = 6e-77
 Identities = 338/401 (84%), Gaps = 0/401 (0%)
 Strand=Plus/Plus

Query  46   GGAACTATCCTCTTTACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCT  105
            ||||| ||| |||||||||||| |||||||| || || ||||| || ||||||||||
Sbjct  100  GGAACAATCTTCTTTACCCAAGAAGGAGATGGTCCTACCACTGTAACTGGAAACATTTCC  159

Query  106  GGCCTCAAGCCTGGGCTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGT  165
            ||||| ||||| |||||||||| ||||| || ||||| ||||| |||||||| || |||
Sbjct  160  GGCCTTAAGCCAGGGCTTCATGGGTTCCACGTCCATGCCCTTGGAGACACAACAAACGGT  219
```

FIG. 5-13

```
Query  166  TGCATGTCAACTGGACCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGAT  225
            ||||||||||||||| ||||| || || ||| |||||||||| |||||  | ||||| |||
Sbjct  220  TGCATGTCAACTGGGCCACACTTTAACCCTTCTGGCAAAGATCATGGTGCCCCTGAGGAT  279

Query  226  GAGACTCGTCATGCTGGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGC  285
            |||| |||||||||||||||||| |||||| |||||| ||| ||||| || |||||| |
Sbjct  280  GAGATTCGTCATGCTGGTGATCTGGGAAATGTCACTGCTGGTGATGATGGCACTGCTAGT  339

Query  286  TTCACAATTGTTGACAAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCT  345
            |||||||| ||||||| || |||||||  |||| | | | || ||||| || |||||
Sbjct  340  TTCACAATTATTGACAAGCATATTCCTCTTTCTGGTCAAAATTCAATCATAGGAAGGGCA  399

Query  346  GTTGTTGTCCATGCAGATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCC  405
            |||||||| |||||||||||||||| ||||||| ||| |||||||| || || ||| ||
Sbjct  400  GTTGTTGTTCATGCAGATCCTGATGATCTTGGCAGGGGAGGACATGAACTCAGTAAAACC  459

Query  406  ACTGGAAATGCTGGTGGCAGGATAGCTTGTGGTATTATTGG  446
            || ||||||||||||||||  |||| || ||||||||||
Sbjct  460  ACCGGAAATGCTGGTGGCAGAGTAGCATGCGGTATTATTGG  500
```

FIG. 5-14

□

> gi|56549630|gb|AY833718.1| Codonopsis lanceolata CuZn superoxide dismutase (SODCc) mRNA, complete cds
Length=799

Score = 289 bits (146), Expect = 4e-75
Identities = 335/398 (84%), Gaps = 0/398 (0%)
Strand=Plus/Plus

```
Query  58   TTTACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGCCT  117
            ||||||||||||||||||||||||||||  || || |||| | ||||||||||  | |||
Sbjct  212  TTTACCCAAGAGGGAGATGGCCCAACTAAAGTTACTGGAAGCCTTTCTGGCCTTCAACCT  271

Query  118  GGGCTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAACT  177
            || | ||| ||||||||||||||||| ||||| ||||||||||||||||||||||||||
Sbjct  272  GGACCTCACGGTTTCCATGTTCATGCCCTTGGTGACACAACCAATGGTTGCATGTCAACT  331

Query  178  GGACCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCAT  237
            || || ||||  |||||||||| ||||| |||||  |||| || || |||| |||||||
Sbjct  332  GGTCCTCATTATAATCCTGCTGGAAAAGAACATGGTGCTCCAGAGGACGAGATTCGTCAT  391
```

FIG. 5-15

```
Query  238  GCTGGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTGTT  297
            |||||||| || || ||| | || || || || ||||| |||||    ||||| || |||
Sbjct  392  GCTGGTGACCTCGGGAATGTTACAGTAGGCGAAGACGGTACTGCAAATTTCACCATCGTT  451

Query  298  GACAAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCCAT  357
            |||||  |||||||| ||  ||||||| || |||||||||| |||||||| |||||||||
Sbjct  452  GACAACCAGATTCCACTATCTGGACCTCATTCTATCATTGGAAGGGCTGTAGTTGTCCAT  511

Query  358  GCAGATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGAAATGCT  417
            || |||||||||| ||||| |||||||| ||||| || ||||||  ||||||||||||
Sbjct  512  GCTGATCCTGATGATCTTGGAAAGGGTGGCCATGAACTCAGCAAAAGCACTGGAAATGCT  571

Query  418  GGTGGCAGGATAGCTTGTGGTATTATTGGCCTTCAAGG  455
            |||||||||| || |||||||| ||||| || |||||
Sbjct  572  GGTGGCAGGATTGCCTGTGGTATCATTGGACTGCAAGG  609
```

□

> gi|74229676|gb|DQ088818.1| Gossypium hirsutum cytoplasmic Cu/ZnSOD mRNA, complete cds

Length=459

Score = 289 bits (146), Expect = 4e-75
Identities = 331/394 (84%), Gaps = 0/394 (0%)
Strand=Plus/Plus

FIG. 5-16

```
Query  62   CCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGCCTGGGC  121
            ||||||| |||||||| |||||||| ||||| || ||| ||||||| || ||||| || |
Sbjct  62   CCCAAGAAGGAGATGGTCCAACTACCGTGACTGGGAACCTTTCTGGTCTTAAGCCGGGAC  121

Query  122  TTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAACTGGAC  181
             | ||||| |||||||||||||| |||||||||||||| || || ||||||||||||||||
Sbjct  122  TCCATGGCTTCCATGTTCATGCCCTTGGGGACACAACTAACGGGTGCATGTCAACTGGAC  181

Query  182  CACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCATGCTG  241
             | ||||| |||||||||||||||||||||||  |||| ||||||||||  || |||||||
Sbjct  182  CCCATTTTAATCCTGCTGGCAAAGAGCATGGTGCTCCNGAAGATGAGAACCGCCATGCTG  241

Query  242  GTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTGTTGACA  301
             ||||||| || ||| |||||||||| ||||| ||   |||  ||||| | ||    ||||
Sbjct  242  GTGATCTAGGNAATGTCACTGTTGGTGATGATGGCTGTGCNAGCTTCTCCATCACCGACA  301

Query  302  AACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCCATGCAG  361
             ||||||||| ||||| || ||| |||| || || || || ||||| ||||||||||||
Sbjct  302  AACAGATTCCNCTCACAGGCCCAAACTCCATTATCGGAAGAGCTGTAGTTGTCCATGCAG  361

Query  362  ATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGAAATGCTGGTG  421
             |||| |||||||||||||||| || |||||||| ||||||  ||| ||||||||||| |
Sbjct  362  ATCCCGATGACCTTGGCAAGGGCGGCCATGAGCTCAGCAAAAGCACAGGAAATGCTGGCG  421
```

FIG. 5-17

```
Query  422  GCAGGATAGCTTGTGGTATTATTGGCCTTCAAGG  455
            ||||  ||||||| |||||||||||  || |||||
Sbjct  422  GCAGAGTAGCTTGCGGTATTATTGGTCTGCAAGG  455
```

□

> gi|73665954|gb|DQ124227.1| Fagus sylvatica putative copper/zinc-superoxide dismutase mRNA, partial cds
Length=388

```
 Score =  283 bits (143),  Expect = 2e-73
 Identities = 284/331 (85%), Gaps = 0/331 (0%)
 Strand=Plus/Plus

Query  57   CTTTACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGCC  116
            |||| ||||||| |||||||||||||||| || || ||||| |||||||||| || ||
Sbjct  58   CTTTGCCCAAGAAGGAGATGGCCCAACTACAGTAACTGGAAATATTTCTGGCCTTAAACC  117

Query  117  TGGGCTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAAC  176
            ||| || ||||| ||||| || |||||||||||||||||| ||||||||||||||||
Sbjct  118  TGGACTCCATGGCTTCCACGTGCATGCTCTTGGGGACACAACAAATGGTTGCATGTCAAC  177
```

FIG. 5-18

```
Query  177  TGGACCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCA  236
            ||||||||||||||||||||||||||||| ||||||  ||||||| |||| || ||||||
Sbjct  178  TGGACCACATTTCAATCCTGCTGGCAAAGGGCATGGTGCTCCTGAGGATGCGAATCGTCA  237

Query  237  TGCTGGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTGT  296
            |||||||||||| |||||| ||| |||||| ||||| || || |   | ||||||||  |
Sbjct  238  TGCTGGTGATCTGGGAAATGTCAATGTTGGTGATGATGGCACAGTCAGTTTCACAATAAT  297

Query  297  TGACAAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCCA  356
            |||||||||||||||| ||   ||| ||| | || || || || ||||||||||||||||
Sbjct  298  TGACAAACAGATTCCACTTTGTGGTCCAAATTCCATTATCGGAAGGGCTGTTGTTGTCCA  357

Query  357  TGCAGATCCTGATGACCTTGGCAAGGGTGGA  387
            || |||||| ||||| ||||||||||||||
Sbjct  358  TGGAGATCCAGATGATCTTGGCAAGGGTGGA  388
```

□

> gi|33340235|gb|AF318938.1| Citrus limon copper/zinc superoxide dismutase mRNA, complete cds
Length=744

FIG. 5-19

```
 Score =  283 bits (143),  Expect = 2e-73
 Identities = 335/399 (83%), Gaps = 0/399 (0%)
 Strand=Plus/Plus

Query  57   CTTTACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGCC  116
            ||||||||| || |||||||| ||||| |||||  | |||| | | ||||| ||||||||
Sbjct  184  CTTTACCCAGGAAGGAGATGGTCCAACAACTGTTTCAGGAAGCCTCTCTGGTCTCAAGCC  243

Query  117  TGGGCTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAAC  176
            ||| | |||||| |||||||||||||||||||| |||||||| |||||||||||||| ||
Sbjct  244  TGGTCCTCATGGATTCCATGTTCATGCTCTTGGAGACACAACAAATGGTTGCATGTCTAC  303

Query  177  TGGACCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCA  236
            |||||| || || || |||||||| ||||| |||||  |||| || ||||| | ||||||
Sbjct  304  TGGACCCCACTTTAACCCTGCTGGAAAAGAACATGGAGCTCCAGAGGATGATAATCGTCA  363

Query  237  TGCTGGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTGT  296
            |||||||||| | |||||| ||| |||| | ||||| || ||||||   || ||| ||||
Sbjct  364  TGCTGGTGATTTAGGAAATGTCAATGTTAGTGATGATGGTACTGCTACTTTTACAGTTGT  423

Query  297  TGACAAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCCA  356
            |||||| ||||||||||  |||||||| | || || ||||| |||||||||| |||||
Sbjct  424  TGACAATCAGATTCCTCTTTCTGGACCAAATTCCATTATTGGAAGGGCTGTTGTAGTCCA  483
```

FIG. 5-20

```
Query  357  TGCAGATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGAAATGC  416
            ||||||||  |||||  ||||||||||  ||  ||||||||  ||||||  ||||||||||||
Sbjct  484  CGCAGATCCCGATGATCTTGGCAAGGGCGGTCATGAGCTGAGCAAAACCACTGGAAATGC  543

Query  417  TGGTGGCAGGATAGCTTGTGGTATTATTGGCCTTCAAGG  455
            |||||||||  |||||||  ||  ||  ||||||||  |||||
Sbjct  544  TGGTGGCAGAGTAGCTTGCGGCATAATTGGCCTCCAAGG  582
```

□

> gi|17385627|dbj|AB062752.1| Bruguiera gymnorhiza SodC mRNA for copper/zinc superoxide dismutase, complete cds
Length=874

```
 Score =  283 bits (143),  Expect = 2e-73
 Identities = 317/375 (84%), Gaps = 0/375 (0%)
 Strand=Plus/Plus

Query  63   CCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGCCTGGGCT  122
            ||||||||||||||||||||||||||  ||  |||||  |||||||||  |||  |  |||||
Sbjct  202  CCAAGAGGGAGATGGCCCAACTACTGTAACTGGAAATGTTTCTGGCCTTAAGTCAGGGCT  261
```

FIG. 5-21

```
Query  123  TCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAACTGGACC  182
             ||||||  ||||||||||||||||||||||||||  ||  ||||||||||||||||||  ||
Sbjct  262  TCATGGCTTCCATGTTCATGCTCTTGGGGACACTACAAATGGTTGCATGTCAACTGGGCC  321

Query  183  ACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCATGCTGG  242
              ||  ||||||||  |  |  ||||||||||||  |  ||||||||  ||||  ||||||||  ||
Sbjct  322  GCACTTCAATCCAGGTAGCAAAGAGCATGGTGCCCCTGAAGACGAGAACCGTCATGCCGG  381

Query  243  TGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTGTTGACAA  302
             |||  ||  ||||||  |  |  |||||  ||||||  ||  |||||    ||||||||   ||||||
Sbjct  382  TGACCTAGGAAATGTAAATGTTGCGGATGATGGCACTGCAACATTCACAATCACTGACAA  441

Query  303  ACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCCATGCAGA  362
              ||||||||||  ||||||||  |  ||  ||  ||||  ||||||||||||||  |||||  ||
Sbjct  442  TCAGATTCCTCTTACTGGACCCAATTCCATTGTTGGAAGGGCTGTTGTTGTTCATGCTGA  501

Query  363  TCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGAAATGCTGGTGG  422
             |||||||||  ||  ||||||||  ||  |||||  |||||||||  ||||||||||||||||||
Sbjct  502  TCCTGATGATCTGGGCAAGGGAGGGCATGAACTTAGCAAAAGCACTGGAAATGCTGGTGG  561

Query  423  CAGGATAGCTTGTGG  437
             ||||  ||||  |||||
Sbjct  562  CAGGGTAGCATGTGG  576
```

FIG. 5-22

□

> gi|52313439|dbj|AB190501.1| Populus alba x Populus tremula var. glandulosa CuZn-SOD mRNA for CuZn-superoxide dismutase, complete cds, clone: PO3024C12
Length=730

Score = 281 bits (142), Expect = 9e-73
Identities = 328/390 (84%), Gaps = 0/390 (0%)
Strand=Plus/Plus

```
Query  57   CTTTACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGCC  116
            ||||||||||||| |||||||| |||||||||||  || |||| | | | ||| || |||||
Sbjct  134  CTTTACCCAAGAAGGAGATGGTCCAACTACTGTAACTGGAAGCCTCTGTGGTCTTAAGCC  193

Query  117  TGGGCTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAAC  176
             ||  |||||||| |||||||||||||| ||||| ||||| || ||||| ||||||||||
Sbjct  194  AGGCCTTCATGGCTTCCATGTTCATGCCCTTGGAGACACCACAAATGGCTGCATGTCAAC  253

Query  177  TGGACCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCA  236
             ||| || ||||| |||||||  ||||||||||||||  | ||||| ||||||| ||||||
Sbjct  254  TGGCCCGCATTTTAATCCTGTAGGCAAAGAGCATGGTGCCCCTGAGGATGAGAATCGTCA  313
```

FIG. 5-23

```
Query  237  TGCTGGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTGT  296
            |||||||||| | |||||| |||||||||| ||||| || || |||    || ||||  |
Sbjct  314  TGCTGGTGATTTGGGAAATGTCACTGTTGGTGATGATGGCACCGCTACTGTCTCAATCAT  373

Query  297  TGACAAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCCA  356
            |||||| |||||||||| ||||||||| | || ||  |||| ||||| |||||||| ||
Sbjct  374  TGACAACCAGATTCCTCTTACTGGACCAAATTCCATTGTTGGAAGGGCAGTTGTTGTTCA  433

Query  357  TGCAGATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGAAATGC  416
            |||||||||||||| |||||||||| |||||||| |||||||||  |||||| |||||
Sbjct  434  TGCAGATCCTGATGATCTTGGCAAGGGAGGACATGAACTTAGCAAAAGCACTGGTAATGC  493

Query  417  TGGTGGCAGGATAGCTTGTGGTATTATTGG  446
            |||||||||  |||| |||||| |||||||
Sbjct  494  TGGTGGCAGAGTAGCATGTGGTGTTATTGG  523
```

□

> gi|52313437|dbj|AB190500.1| Populus alba x Populus tremula var. glandulosa CuZn-SOD mRNA for CuZn-superoxide dismutase, complete cds, clone: PO3023E02
Length=725

FIG. 5-24

```
 Score =  281 bits (142),  Expect = 9e-73
 Identities = 328/390 (84%), Gaps = 0/390 (0%)
 Strand=Plus/Plus

Query  57   CTTTACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGCC  116
            ||||||||||||| |||||||| |||||||||||  || ||||  |  |  |  |||  ||  |||||
Sbjct  154  CTTTACCCAAGAAGGAGATGGTCCAACTACTGTAACTGGAAGCCTCTGTGGTCTTAAGCC  213

Query  117  TGGGCTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAAC  176
             ||  |||||||| |||||||||||||| |||||  |||||  ||  |||||  ||||||||||
Sbjct  214  AGGCCTTCATGGCTTCCATGTTCATGCCCTTGGAGACACCACAAATGGCTGCATGTCAAC  273

Query  177  TGGACCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCA  236
             |||  ||  |||||  |||||||  ||||||||||||||  |  |||||  |||||||  ||||||
Sbjct  274  TGGCCCGCATTTTAATCCTGTAGGCAAAGAGCATGGTGCCCCTGAGGATGAGAATCGTCA  333

Query  237  TGCTGGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTGT  296
             |||||||||  |  ||||||  |||||||||  |||||  ||  ||  |||     ||  ||||   |
Sbjct  334  TGCTGGTGATTTGGGAAATGTCACTGTTGGTGATGATGGCACCGCTACTGTCTCAATCAT  393

Query  297  TGACAAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCCA  356
             ||||||  ||||||||||  |||||||||  |  ||  ||  ||||  |||||  ||||||||  ||
Sbjct  394  TGACAACCAGATTCCTCTTACTGGACCAAATTCCATTGTTGGAAGGGCAGTTGTTGTTCA  453
```

FIG. 5-25

```
Query  357  TGCAGATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGAAATGC  416
            ||||||||||||||| |||||||||| |||||||| |||||||||  |||||| |||||
Sbjct  454  TGCAGATCCTGATGATCTTGGCAAGGGAGGACATGAACTTAGCAAAAGCACTGGTAATGC  513

Query  417  TGGTGGCAGGATAGCTTGTGGTATTATTGG  446
            |||||||||  ||||  ||||||  |||||||
Sbjct  514  TGGTGGCAGAGTAGCATGTGGTGTTATTGG  543
```

□

> gi|2708805|gb|AF037359.1|AF037359 Paulownia kawakamii superoxide dismutase (SOD5) mRNA, complete cds
Length=794

Score = 276 bits (139), Expect = 6e-71
Identities = 352/423 (83%), Gaps = 0/423 (0%)
Strand=Plus/Plus

```
Query  30   CAGTGAGGGTGTTGCTGGAACTATCCTCTTTACCCAAGAGGGAGATGGCCCAACTACTGT  89
            |||||||||||||  ||| || |||  ||| ||||| || |||||||| ||||| |||||
Sbjct  117  CAGTGAGGGTGTTAGTGGCACCATCTACTTCACCCAGGAAGGAGATGGTCCAACAACTGT  176
```

FIG. 5-26

```
Query  90   GACCGGAAACATTTCTGGCCTCAAGCCTGGGCTTCATGGTTTCCATGTTCATGCTCTTGG  149
Sbjct  177  TACTGGAAACGTTTCTGGCCTTAAGCCTGGACCCCATGGCTTTCATGTGCATGCCCTTGG  236

Query  150  GGACACAACCAATGGTTGCATGTCAACTGGACCACATTTCAATCCTGCTGGCAAAGAGCA  209
Sbjct  237  TGACACCACCAATGGTTGTTTGTCAACTGGACCTCACTTCAATCCTGCTGGCAAAGAGCA  296

Query  210  TGGGTCTCCTGAAGATGAGACTCGTCATGCTGGTGATCTTGGAAATATCACTGTTGGGGA  269
Sbjct  297  TGGAGCTCCTGATGATGAGGTTCGCCATGCTGGTGACCTTGGGAATGTCACAGTTGGAGA  356

Query  270  TGACGGAACTGCTTGCTTCACAATTGTTGACAAACAGATTCCTCTCACTGGACCACACTC  329
Sbjct  357  AGATGGCACTGCTGCTTTCACTATTGTTGACAAGCAGATACCACTTACAGGACCACATTC  416

Query  330  TATCATTGGTAGGGCTGTTGTTGTCCATGCAGATCCTGATGACCTTGGCAAGGGTGGACA  389
Sbjct  417  CATAATTGGAAGAGCTGTAGTTGTTCATGCTGATCCTGATGATCTTGGAAAGGGTGGACA  476

Query  390  TGAGCTTAGCAAATCCACTGGAAATGCTGGTGGCAGGATAGCTTGTGGTATTATTGGCCT  449
Sbjct  477  TGAACTGAGCAAAACCACTGGAAATACTGGTGGAAGAGTTGCTTGTGGTATCAATGGCCT  536
```

FIG. 5-27

```
Query  450  TCA  452
            |||
Sbjct  537  TCA  539
```

□

> gi|39840778|emb|AJ428575.2|OEU428575 Olea europaea Cu/Zn super-oxide dismutase (ole e 5 allergen)

```
Length=714
 Score =  272 bits (137),  Expect = 9e-70
 Identities = 331/393 (84%), Gaps = 2/393 (0%)
 Strand=Plus/Plus

Query  61   ACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTCAAGCCTGGG  120
            ||||||||  ||||||||  ||||||||||  ||  ||||||  ||||||||||  ||||||||
Sbjct  61   ACCCAAGAAGGAGATGGTCCAACTACTGTTACTGGAAACCTTTCTGGCCTTAAGCCTGGA  120

Query  121  CTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATGTCAACTGGA  180
            ||||||||  ||  |||||  ||  ||  |||||  |||||  ||||||||  ||  ||||||||||||
Sbjct  121  CTTCATGGCTTTCATGTCCACGCCCTTGGTGACACCACCAATGGCTGTATGTCAACTGGA  180
```

FIG. 5-28

```
Query  181  CCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCATGCT  240
            ||  ||||||||||||||  |||  ||||||||||  |  ||||  ||||||||  |||||||||
Sbjct  181  CCTCATTTCAATCCTGTTGGGAAAGAGCATGGTGCACCTGGAGATGAGAACCGTCATGCT  240

Query  241  GGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCT-TCACAATTGTTGA  299
            ||||||||||  ||||||||  |||||  ||  ||  ||  ||  ||||  |||  ||||||||
Sbjct  241  GGTGATCTTGGTAATATCACAGTTGGCGAAGATGGCACCGC-TGCTATCAACATTGTTGA  299

Query  300  CAAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCCATGC  359
            |||  |||||  |||||  ||  ||||||||  ||  ||  |||||  ||  ||  ||  |||||||||  |
Sbjct  300  CAAGCAGATACCTCTTACAGGACCACATTCCATAATTGGAAGAGCAGTAGTTGTCCATTC  359

Query  360  AGATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGAAATGCTGG  419
            ||||||||||||  |||||  |  ||||||  |||||  ||  |||||   ||||||||||||||
Sbjct  360  AGATCCTGATGATCTTGGAAGGGGTGGTCATGAACTGAGCAAGAGCACTGGAAATGCTGG  419

Query  420  TGGCAGGATAGCTTGTGGTATTATTGGCCTTCA  452
            |||  ||   |  ||||||||||  ||||||||||
Sbjct  420  TGGAAGAGTTGCTTGTGGTATCATTGGCCTTCA  452
```

FIG. 5-29

□

> gi|13751865|gb|AF355460.1|AF355460 U Solanum tuberosum Cu/Zn-superoxide dismutase mRNA, partial cds
Length=617

Score = 268 bits (135), Expect = 1e-68
Identities = 330/395 (83%), Gaps = 0/395 (0%)
Strand=Plus/Plus

```
Query  52   ATCCTCTTTACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTC  111
            ||||||||  ||  |||||  |||||||  |||||  ||  ||  |  |||||  ||||||||||
Sbjct  30   ATCCTCTTCACTCAAGATGGAGATGCTCCAACCACAGTTAATGGAAATATTTCTGGCCTA  89

Query  112  AAGCCTGGGCTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAATGGTTGCATG  171
            ||  |||||  ||||||||  ||||||||  |||||  |||||  ||  ||  ||  |||||  ||||||
Sbjct  90   AAACCTGGACTTCATGGCTTCCATGTCCATGCCCTTGGTGATACCACAAATGGCTGCATG  149

Query  172  TCAACTGGACCACATTTCAATCCTGCTGGCAAAGAGCATGGGTCTCCTGAAGATGAGACT  231
            |||||  ||||||||||  ||||||||||||  ||  ||||||||  ||||||||||||||
Sbjct  150  TCAACAGGACCACATTACAATCCTGCTGGTAAGGAGCATGGTGCTCCTGAAGATGAGGTG  209

Query  232  CGTCATGCTGGTGATCTTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACA  291
            ||||||||||||||||||||  ||  |||||  |||||  ||  ||  ||  |||||  |  ||  ||
Sbjct  210  CGTCATGCTGGTGATCTTGGTAACATCACAGTTGGAGAAGATGGTACTGCATCTTTTACT  269
```

FIG. 5-30

```
Query  292  ATTGTTGACAAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTT  351
             |||   ||||| ||||||||||||||||  |||| || |||||||| || |||||||||
Sbjct  270  ATTACCGACAAGCAGATTCCTCTCACTGGTTCACAATCCATCATTGGAAGAGCTGTTGTT  329

Query  352  GTCCATGCAGATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGA  411
             || ||||| |||||||||| ||||| ||||| |||||||||| || |||  |||||||
Sbjct  330  GTTCATGCTGATCCTGATGATCTTGGAAAGGGAGGACATGAGCTCAGTAAAAGCACTGGA  389

Query  412  AATGCTGGTGGCAGGATAGCTTGTGGTATTATTGG  446
             |||||||| || ||||| ||||||||||||||||
Sbjct  390  AATGCTGGCGGAAGGATTGCTTGTGGTATTATTGG  424
```

FIG. 5-31

Detail of SEQ ID No. 1
Organization Applicant

Street : Rafi marg,
City : New Delhi
State : Delhi
Country : India
PostalCode : -110001
PhoneNumber :
FaxNumber :
EmailAddress :

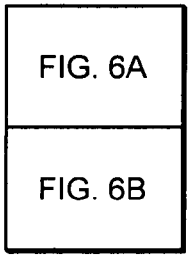
<110> OrganizationName : COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH

Application Project

<120> Title : SUPEROXIDE DISMUTASE (SOD) GENE AND A METHOD OF IDENTIFYING AND CLONING THEREOF
<130> AppFileReference : 0038NF2006
<140> CurrentAppNumber :
<141> CurrentFilingDate : 2006-03-31

FIG. 6A

FIG. 6



Sequence

<213> OrganismName : Potentilla atrosanguinea

<400> PreSequenceString :

MAKGVAVLSS SEGVAGTILF TQEGDGPTTV TGNISGLKPG LHGFHVHALG DTTNGCMSTG 60
PHFNPAGKEH GSPEDETRHA GDLGNITVGD DGTACFTIVD KQIPLTGPHS IIGRAVVVHA 120
DPDDLGKGGH ELSKSTGNAG GRIACGIIGL QG 152

<212> Type : PRT

<211> Length : 152

SequenceName : Polypeptide sequence of SOD gene SEQ ID NO. 1

SequenceDescription :

FIG. 6B

FIG. 7

FIG. 7A

FIG. 7B

Detail of SEQ ID No. 2
Organization Applicant

Street : Rafi marg,
City : New Delhi
State : Delhi
Country : India
PostalCode : -110001
PhoneNumber :
FaxNumber :
EmailAddress :

<110> OrganizationName : COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH

Application Project

<120> Title : SUPEROXIDE DISMUTASE (SOD) GENE AND A METHOD OF IDENTIFYING AND CLONING THEREOF
<130> AppFileReference : 0038NF2006
<140> CurrentAppNumber :
<141> CurrentFilingDate : 2006-03-31

FIG. 7A

Sequence

<213> OrganismName : Potentilla atrosanguinea

<400> PreSequenceString :

acgggggggg gactgaaata aatagagagg gtcatagtca catttgcatt taggtatctg 60

attccattca caaacctcca actcccacct ctctctctat ttctcttcat cttcatcatc 120

ttagggtgca ctgagatcac tttgaaacat ggcaaagggc gttgctgtac ttagctccag 180

tgagggtgtt gctggaacta tcctctttac ccaagaggga gatggcccaa ctactgtgac 240

cggaaacatt tctggcctca agcctgggct tcatggtttc catgttcatg ctcttgggga 300

cacaaccaat ggttgcatgt caactggacc acatttcaat cctgctggca aagagcatgg 360

gtctcctgaa gatgagactc gtcatgctgg tgatcttgga aatatcactg ttggggatga 420

cggaactgct tgcttcacaa ttgttgacaa acagattcct ctcactggac cacactctat 480

cattggtagg gctgttgttg tccatgcaga tcctgatgac cttggcaagg gtggacatga 540

gcttagcaaa tccactggaa atgctggtgg caggatagct tgtggtatta ttggccttca 600

aggatgaact ggaccaggga gcgaaacaca ggcatcttgt tgaattaaaa cttgagatat 660

tagcgaactc ttcggaattg agtattgaaa caaggaatac atttgtcatt accaatacgt 720

ttggcttaga cctgtattct gtatctcaat agttttctgt gtggttgttt gacagttatt 780

tgtgctcagg ctatttcaaa gggataaaca cagtaacttt cttgctttga caaaaaaaaa 840

aaaaaaaaaa aaaaaa 856

<212> Type : DNA

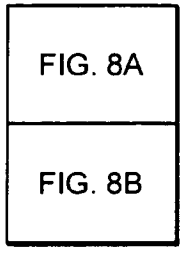
<211> Length : 856

SequenceName : Full length cDNA SOD gene of SEQ ID No. 2

SequenceDescription :

FIG. 7B

FIG. 8



detail of SEQ ID No. 3
Organization Applicant

Street : Rafi marg,
City : New Delhi
State : Delhi
Country : India
PostalCode : -110001
PhoneNumber :
FaxNumber :
EmailAddress :

<110> OrganizationName : COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH

Application Project

<120> Title : SUPEROXIDE DISMUTASE (SOD) GENE AND A METHOD OF IDENTIFYING AND CLONING THEREOF
<130> AppFileReference : 0038NF2006
<140> CurrentAppNumber :
<141> CurrentFilingDate : 2006-03-31

FIG. 8A

Sequence

<213> OrganismName : Potentilla atrosanguinea

<400> PreSequenceString :

atggcaaagg gcgttgctgt acttagctcc agtgagggtg ttgctggaac tatcctcttt 60

acccaagagg gagatggccc aactactgtg accggaaaca tttctggcct caagcctggg 120

cttcatggtt tccatgttca tgctcttggg gacacaacca atggttgcat gtcaactgga 180

ccacatttca atcctgctgg caaagagcat gggtctcctg aagatgagac tcgtcatgct 240

ggtgatcttg gaaatatcac tgttggggat gacggaactg cttgcttcac aattgttgac 300

aaacagattc ctctcactgg accacactct atcattggta gggctgttgt tgtccatgca 360

gatcctgatg accttggcaa gggtggacat gagcttagca aatccactgg aaatgctggt 420

ggcaggatag cttgtggtat tattggcctt caaggatga 459

<212> Type : DNA

<211> Length : 459

SequenceName : Coding sequence of potentialla SOD gene of SEQ ID NO. 3

SequenceDescription :

FIG. 8B

FIG. 9

FIG. 9A

FIG. 9B

Detail of SEQ ID No. 4
Organization Applicant

Street : Rafi marg,
City : New Delhi
State : Delhi
Country : India
PostalCode : -110001
PhoneNumber :
FaxNumber :
EmailAddress :

<110> OrganizationName : COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH

Application Project

<120> Title : SUPEROXIDE DISMUTASE (SOD) GENE AND A METHOD OF IDENTIFYING AND CLONING THEREOF
<130> AppFileReference : 0038NF2006
<140> CurrentAppNumber :
<141> CurrentFilingDate : 2006-03-31

FIG. 9A

Sequence

<213> OrganismName : Potentilla atrosanguinea

<400> PreSequenceString :

caagagggag atggcccaac tactgtgacc ggaaacattt ctggcctcaa gcctgggctt 60

catggtttcc atgttcatgc tcttggggac acaaccaatg gttgcatgtc aactggacca 120

catttcaatc ctgctggcaa agagcatggg tctcctgaag atgagactcg tcatgctggt 180

gatcttggaa atatcactgt tggggatgac ggaactgctt gcttcacaat tgttgacaaa 240

cagattcctc tcactggacc acactctatc attggtaggg ctgttgttgt ccatgcagat 300

cctgatgacc ttggcaaggg tggacatgag cttagcaaat ccactggaaa tgctggtggc 360

aggat 365

<212> Type : DNA

<211> Length : 365

SequenceName : Positive cDNA clone of SEQ ID No. 4

SequenceDescription :

FIG. 9B

FIG. 10

FIG. 10A

FIG. 10B

Detail of SEQ ID No. 4

Organization Applicant

Street : Rafi marg,
City : New Delhi
State : Delhi
Country : India
PostalCode : -110001
PhoneNumber :
FaxNumber :
EmailAddress :

<110> OrganizationName : COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH

Application Project

<120> Title : SUPEROXIDE DISMUTASE (SOD) GENE AND A METHOD OF IDENTIFYING AND CLONING THEREOF
<130> AppFileReference : 0038NF2006
<140> CurrentAppNumber :
<141> CurrentFilingDate : 2006-03-31

FIG. 10A

Sequence

<213> OrganismName : Potentilla atrosanguinea

<400> PreSequenceString :

atggcaaagg gcgttgctgt actt 24

<212> Type : DNA

<211> Length : 24

SequenceName : Primer Sequence SEQ ID No. 5(a) Forward Primer

SequenceDescription :

Sequence

<213> OrganismName : Potentilla atrosanguinea

<400> PreSequenceString :

tcatccttga aggccaataa tacca 25

<212> Type : DNA

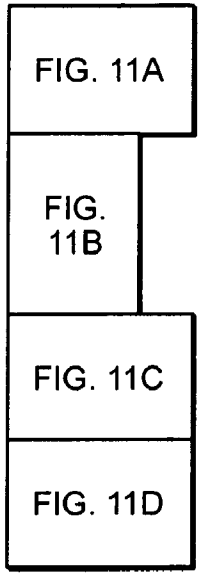
<211> Length : 25

SequenceName : Primer sequence SEQ ID No. 5(b) : Reverse primer

SequenceDescription :

FIG. 10B

FIG. 11



SEQ ID No. 6
Organization Applicant

Street : Rafi marg,
City : New Delhi
State : Delhi
Country : India
PostalCode : -110001
PhoneNumber :
FaxNumber :
EmailAddress :
<110> OrganizationName : COUNCIL OF SCIENTIFIC & INDUSTRIAL RESEARCH

Application Project

<120> Title : SUPEROXIDE DISMUTASE (SOD) GENE AND A METHOD OF IDENTIFYING AND CLONING THEREOF
<130> AppFileReference : 0038NF2006
<140> CurrentAppNumber :
<141> CurrentFilingDate : 2006-03-31

FIG. 11A

Sequence

<213> OrganismName : Potentilla atrosanguinea
<400> PreSequenceString :
ccagtggatt tgctaagctc atgtcca 27
<212> Type : DNA
<211> Length : 27
SequenceName : Primer Sequence of SEQ ID No. 6(a) GSP1:Forward primer
SequenceDescription :

Sequence

<213> OrganismName : Potentilla atrosanguinea
<400> PreSequenceString :
gtcatcaggg tctgcatgga caacaac 27
<212> Type : DNA
<211> Length : 27
SequenceName : Primer sequence of SEQ ID No. 6(b) NES1: Reverse Primer
SequenceDescription :

Sequence

<213> OrganismName : Potentilla atrosanguinea
<400> PreSequenceString :
atggttgcat gtcaactgga ccacatt 27
<212> Type : DNA
<211> Length : 27
SequenceName : Primer Sequence of SEQ ID No. 6(c) GSP2: Forward Primer
SequenceDescription :

Sequence

<213> OrganismName : Potentilla atrosanguinea
<400> PreSequenceString :
ttgcatgtca actggaccac atttcaa 27
<212> Type : DNA
<211> Length : 27
SequenceName : Primer sequence of SEQ ID No. 6(d) NES2: Reverse Primer
SequenceDescription :

FIG. 11B

Sequence

<213> OrganismName : Potentilla atrosanguinea

<400> PreSequenceString :

aagcagtggt atcaacgcag agtacgcggg 30

<212> Type : DNA

<211> Length : 30

SequenceName : Primer Sequence of SEQ ID No. 6(e): SMART II A Oligonucleotide

SequenceDescription :

Sequence

<213> OrganismName : Potentilla atrosanguinea

<400> PreSequenceString :

aagcagtggt atcaacgcag agtactnn 28

<212> Type : DNA

<211> Length : 28

SequenceName : Primer sequence of SEQ ID No. 6(f): 3`- RACE CDS Primer A (3`-CDS):

SequenceDescription :

FIG. 11C

SUPEROXIDE DISMUTASE GENE FROM POTENTILLA ATROSANGUINEA AND ITS EXPRESSION IN HETEROLOGOUS SYSTEM

Abstract:

The present invention provides a superoxide dismutase gene from *Potentilla atrosanguinea*, a construct containing the gene coding for superoxide dismutase and transformed *E.coli* producing the SOD protein.

FIG. 11D

SUPEROXIDE DISMUTASE (SOD) GENE AND A METHOD OF IDENTIFYING AND CLONING THEREOF

FIELD OF INVENTION

[0001] The present invention relates to a Superoxide dismutase (SOD). Superoxide dismutase (SOD) cDNA of SEQ ID No. 2 obtained from *Potentilla atrosanguinea* containing coding gene sequence of SEQ ID No. 3 which codes for a polypeptide of SEQ ID No. 1 having Superoxide dismutase enzyme activity.

[0002] Further, it also relates to a set of primers useful for the amplification of Superoxide dismutase (SOD) gene coding cDNA of SEQ ID No. 3, wherein

```
Forward primer    5'-ATGGCAAAGGGCGTTGCTGTACTT-3'
and;

Reverse primer    5'-TCATCCTTGAAGGCCAATAATACCA-3'
```

[0003] More particularly, it relates to a method of identifying and cloning of Superoxide dismutase (SOD) gene of SEQ ID NO 3, which on expression gives a Superoxide dismutase enzyme (EC 1.15.1.1) with the characteristics disclose in U.S. Pat. No. 6,485,950.

BACKGROUND AND PRIOR ART REFERENCES TO THE INVENTION

[0004] SOD is a ubiquitous enzyme present in plants, animals and microbes, which protects them against oxidative damage caused by superoxide radical (hereinafter, referred to O_2^-). The enzyme dismutates superoxide radical into hydrogen peroxide and oxygen as per the following redox reaction:

$$2O_2^{-}\cdot+2H^+=H_2O_2+O_2$$

[0005] Thus, SOD has implications in all those reactions, wherein $O_2^{-}\cdot$ is produced in the amount leading to cellular injury. According to the U.S. Pat. No. 6,485,950, we have extracted an autoclavable superoxide dismutase from *Potentilla* that could be autoclaved and shows activity at sub-zero temperature. Due to prevalence of *Potentilla* at difficult to access location of high altitude, and industrial implications of SOD as mentioned in our U.S. Pat. No. 6,485,950, it was essential to develop a system for the production of SOD of *Potentilla* in *E.coli* so as to obtain the SOD when desired.

[0006] Below is given state of the art knowledge in relation to isolation of SOD genes from various sources and their expression in *E.coli,* to produce SOD in recoverable quantities.

[0007] Reference may be made to document (1) by Wang, Z., He, Z., Shen, Q., Gu, Y., Li, S. and Yuan, Q. (J. of Chromatography B, 2005. 826: 114-121) wherein Cu/Zn SOD gene from *Cordyceps militaris* was overexpressed in *E. coli.*

[0008] Yet another reference may be made to document (2) by Liu, W., Zhu, R. H., Li, G. P., and Wang, D. C. (Protein Expr. Purif. 2002. 25: 379-388) wherein production of high yield of recombinant duck Cu/Zn SOD was achieved in *E. coli.*

[0009] Reference may be made to yet another document (3) by Pan, S. M., Hwang, G. B., and Liu, H. C. (Bot. Bull. Acad. Sin. 1999. 40: 275-281) wherein over-expression and characterization of cytosolic Cu/Zn SOD from rice in *E. coli* was achieved.

[0010] Reference may be made to document (4) by Hartman, J. R., Geller, T., Yavin, Z., Bartfeld, D., Kanner, D., Aviv, H., and Gorecki, M. (Proc. Natl. Acad. Sci. USA. 1986. 83: 7142-7146) wherein high-level expression of enzymatically active human Cu/Zn SOD was reported in *E. coli.*

[0011] Reference may be made to document (5) by Ken, C. F., Lin, C. T., Shaw, J. F., and Wu, J. L. (Marine Biotech. 2003. 5: 167-173) wherein the Cu/Zn SOD from zebrafish was over-expressed in *E. coli* and the active enzyme was purified.

[0012] Reference may be made to document (6) by Kim, T. S., Jung, Y., Na, B. K., Kim, K. S., and Chung, P. R. (Infect. Immun. 2000. 68: 3941-3948) wherein the Cu/Zn SOD gene from *Fasciola hepatica* was cloned and expressed in *E. coli.*

[0013] The drawbacks are:

[0014] 1. There is no SOD gene that is isolated from *Potentilla,* a source of Cu/ZnSOD that is autoclavable and functions at sub-zero temperature.

[0015] 2. There is no SOD gene that is isolated from *Potentilla* and made to express in *E. coli.*

[0016] 3. There is no SOD gene that is made to express in *E.coli* leading to SOD protein that is shown to be autoclavable.

[0017] 4. There is no SOD gene that is made to express in *E.coli* leading to SOD protein that is shown to function at sub-zero temperature.

Comparative data of present SOD with other known SOD

Present invention	Prior art
The maximum thermostability of SOD described so far is at 80° C.	The maximum thermostability of SOD is 37° C. to 50° C. reference from Bueno P., Verla, J., Gallego, G. G., and Rio del A. L. (Plant Physiol. 1995. 108: 1151-1160) wherein the thermostability of Cu/Zn SOD

-continued

Comparative data of present SOD with other known SOD

Present invention	Prior art
	isolated from the cotyledon of water melon has been shown, SOD activity reduced: (a) by 40% after 4 hour of incubation at 50.° C.; (b) by 50% after 15 minute of incubation at70° C.; (c) by 80% after 60 minute of incubation at 80° C.; and (d) by 100% after 15 minute of incubation at 100° C. Reference may be made to Document by Miyata, K., Maejima, K., and Tomoda, K. (U.S. Pat. No. 4,563,349; Jan. 7, 1986) wherein SOD has been reported from a microorganism belonging to genus *Serratia* having the thermostability characters as follows: (a) Stable at 37° C. for 60 minutes; Inactivated by 50% when incubated at 50-60° C. for60 minutes; and Inactivated by 100% when incubated at 80° C. for 5 minutes.
stability without adding an external stabilizer [the addition of hydrogen peroxide trapping agent, polyols, and sugars etc. are required to stabilise the enzyme from other sources such as germinated plant seeds	External stabilizer is required to enhance the stability of the product contains this enzyme. Reported SODs do not retain their activity at ambient temperature unless stabilized by the addition of polyols, sugars or any other stabilizing agent (Bresson-Rival; Delphine; Boivin; Patrick; Linden; Guy; Perrier; Erric; Humbert; Gerard; 1999; U. S. Pat. No.
Wide range of temperature functionality from sub-zero to above 50.degree. C. temperature which would immensely enhance the utility of the enzyme and its products and be safer for use for humans.	Temperature range for SOD activity has been reported between 5 to 45. degree. C. Hakam, N. and Simon, J. P. 1996. Physiol. Plant. 97: 209-216). However, thermostability and lower temperature for catalyzing dismutation of O.sub.2.sup.−. are not reported for the same enzyme.
Present enzyme is autoclavable. When SOD is to be injected in the body, a sterile composition would be needed and for that an autoclavable SOD would be an ideal one. Moreover, in reperfusion applications and storage of organs at low temperature, an autoclavable SOD would be required which can function efficiently at low temperature as well. Apart from the use of autoclaved SOD in Pharmaceuticals and medical fields, sterile SOD will also be a choice in the cosmetic and food industry.	There is no report for autoclavable SOD.

OBJECTS OF THE INVENTION

[0018] The main object of the invention is to provide a superoxide dismutase (SOD) Superoxide dismutase (SOD) cDNA of SEQ ID No. 2 obtained from *Potentilla atrosanguinea* containing coding gene sequence of SEQ ID No. 3 which codes for a polypeptide of SEQ ID No. 1 having Superoxide dismutase enzyme activity

[0019] Another object of the present invention is to provide a set of primers useful for the amplification of Superoxide dismutase (SOD) gene coding cDNA of SEQ ID No. 3, wherein

```
Forward primer     5'-ATGGCAAAGGGCGTTGCTGTACTT-3'
and;

Reverse primer     5'-TCATCCTTGAAGGCCAATAATACCA-3'
```

[0020] Further, another object of the present invention is to provide a method of identifying and cloning of superoxide dismutase (SOD) gene of SEQ ID NO 3, which on expression gives a superoxide dismutase enzyme (EC 1.15.1.1) with the characteristics disclose in U.S. Pat. No. 6,485,950.

[0021] Yet another object of the present invention is to provide a gene responsible for autoclavable superoxide dismutase from *Potentilla.*

[0022] Still another object of the present invention is to provide a gene responsible for autoclavable superoxide dismutase from *Potentilla* that is also functional at sub-zero temperature.

[0023] Still another object of the present invention is to provide a recombinant gene of SOD, which shows activity upon autoclaving and also shows activity at low temperature, in a plasmid vector leading to a new vector which carries the nucleotide sequence synthesizing the said SOD.

[0024] Still another object of the present invention is to transform bacterial host *E. coli* with the above said recombinant plasmid vector for expression of the SOD gene in the bacterial host.

BRIEF DESCRIPTION OF FIGURES

[0025] FIG. **1** represents effect of assay temperature on SOD activity. *Potentilla* SOD expressing in *E. coli* was purified and assayed before and after autoclaving at different temperatures.

[0026] FIG. **2** represents comparison of the nucleotide sequence of the *Potentilla* Cu/Zn SOD with sequences from other plant species. Regions of complete homology are indicated with asterisks.

[0027] FIG. **3** represents comparison of the deduced amino acid sequence of the *Potentilla* Cu/Zn SOD with sequences from other plant species. Regions of complete homology are indicated with asterisks.

[0028] FIG. **4**(A) represents expression and purification of *Potentilla* SOD in *E. coli.* C, Control; I, Protein induced by IPTG; P, Purified SOD. The gel was stained by silver staining. (B). Activity staining of the gel to depict the activity of purified SOD. P, Purified SOD.

[0029] FIG. **5** represents the result of alignment of present sod gene with the sod gene of other plant species

[0030] FIG. **6** represents the details of Polypeptide sequence of SOD gene SEQ ID NO. 1.

[0031] FIG. **7** represents the details of full length cDNA SOD gene of SEQ ID No. 2

[0032] FIG. **8** represents the details of coding sequence of potentialla SOD gene of SEQ ID NO. 3

[0033] FIG. **9** represents the details of positive cDNA clone of SEQ ID No. 4

[0034] FIG. **10** represents primer Sequence SEQ ID No. 5(a) Forward Primer and Primer sequence SEQ ID No. 5(b): Reverse primer.

[0035] FIG. **10** represents Details of primers used for RACE

[0036] (a) Primer Sequence of SEQ ID No. 6(a) GSP1:Forward primer.

[0037] (b) Primer sequence of SEQ ID No. 6(b) NES1: Reverse Primer

[0038] (c) Primer Sequence of SEQ ID No. 6(c) GSP2: Forward Primer

[0039] (d) Primer sequence of SEQ ID No. 6(d) NES2: Reverse Primer

SUMMARY OF THE INVENTION

[0040] The present invention provides superoxide dismutase gene from *Potentilla atrosanguinea* and its expression in heterologous system and comprises of a construct which carries the coding nucleotide sequence of SEQ ID 3 which is responsible fore synthesis of said SOD and transformed *E.coli* producing the SOD protein. This SOD protein is autoclavable and also functions at sub-zero temperature.

DETAILED DESCRIPTION OF THE INVENTION

[0041] Accordingly, the present invention provides a superoxide dismutase (SOD) cDNA of SEQ ID No. 2 obtained from *Potentilla atrosanguinea,* wherein the said cDNA comprises 856 nucleotide bases.

[0042] In an embodiment of the present invention, the said cDNA has entire coding sequence along with pre- and post-coding sequences.

[0043] The present invention also provides a superoxide dismutase (SOD) gene coding cDNA of SEQ ID No. 3, wherein the said coding cDNA comprises 459 nucleotide bases.

[0044] Further, it also provides a superoxide dismutase (SOD) polypeptide of SEQ ID No. 1, wherein the said polypeptide comprises 152 amino acids.

[0045] In an embodiment of the present invention, the said polypeptide is autoclavable.

[0046] In another embodiment of the present invention, the said polypeptide is functional at temperature range of <−10° C. to +80° C.

[0047] The present invention further provides a set of primers useful for the amplification of Superoxide dismutase (SOD) gene coding cDNA of SEQ ID No. 3, wherein

```
Forward primer    5'-ATGGCAAAGGGCGTTGCTGTACTT-3'
and;

Reverse primer    5'-TCATCCTTGAAGGCCAATAATACCA-3'
```

[0048] 1. Further, it provides A method of identifying and cloning of superoxide dismutase (SOD) gene of SEQ ID NO 3 which codes for a polypeptide of SEQ ID No. 1 having Superoxide dismutase enzyme activity, wherein the said method comprising the steps of:

[0049] a) isolating the mRNA from leaves of *potentialla;*

[0050] b) synthesizing the cDNA from mRNA as obtained from step (a);

[0051] c) constructing a cDNA library of the DNA of *potentilla* followed by the cloning of the cDNA obtained from step (b) in a suitable vector preferably in bacteriophage;

[0052] d) screening the said library obtained from step (c) followed by the primary, secondary and tertiary screening for identification of positive cDNA clones;

[0053] e) isolating the DNA from positive cDNA clones obtained from step (d);

[0054] f) amplifying the said DNA using the primers comprising:

```
Forward Primer:   5'-GTTGTAAAACGACGTGCCAGT-3'

Reverse Primer:   5'-CACAGGAAACAGCTATGACC-3';
```

[0055] g) amplifying the ends of cDNA obtained from step (e) through rapid amplification of cDNA ends technique (RACE) using two set of primers to get the full length desired Superoxide dismutase (SOD) DNA of SEQ ID NO. 2 wherein the said primers comprising:

```
Forward Primer: 5'-CCAGTGGATTTGCTAAGCTCATGTCCA-3'

Reverse Primer: 5'-GTCATCAGGGTCTGCATGGACAACAAC-3'

Forward Primer: 5'-ATGGTTGCATGTCAACTGGACCACATT-3'

Reverse Primer: 5'-TTGCATGTCAACTGGACCACATTTCAA-3'
```

[0056] g) amplifying the ends of cDNA obtained from step (e) through rapid amplification of cDNA ends technique (RACE) using different set of primers to get the full length desired Superoxide dismutase (SOD) DNA of SEQ ID NO. 2 wherein the said primers comprising:

```
Forward Primer (GSP1):
5'-CCAGTGGATTTGCTAAGCTCATGTCCA-3'

Reverse Primer (NES1):
5'-GTCATCAGGGTCTGCATGGACAACAAC-3'

Forward Primer (GSP2):
5'-ATGGTTGCATGTCAACTGGACCACATT-3'

Reverse Primer (NES2):
5'-TTGCATGTCAACTGGACCACATTTCAA-3'

SMART II A Oligonucleotide:
5'AAGCAGTGGTATCAACGCAGAGTACGCGGG-3'

3'-RACE CDS Primer A (3'-CDS):
5'AAGCAGTGGTATCAACGCAGAGTAC(T)30N-1N-3'

5'-RACE CDS Primer (5'-CDS)
5'-(T)25N-1N-3'

Universel Primer Mix A (UPM):Long:
5'TAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3'

Universel Primer Mix A (UPM):Short:
5'-CTAATACGACTCACTATAGGGC-3'

Nested Universal Primer A (NUP):
5'-AAGCAGTGGTATCAACGCAGAGT-3'
```

[0057] h) amplifying the coding sequence of Superoxide dismutase (SOD) of SEQ ID No. 3 using a set of primers designed from start and stop codon of full length desired Superoxide dismutase (SOD) DNA of SEQ ID NO. 2 wherein the said primers have the following sequences:

```
Forward Primer:   5'-ATGGCAAAGGGCGTTGCTGTACTT-3'

Reverse Primer:   5'-TCATCCTTGAAGGCCAATAATACCA-3'
```

[0058] i) cloning the amplified product obtained from step (g) into pQE 30 expression vector followed by the transformation it into competent *E.coli* cells to get an expression construct;

[0059] j) isolating the plasmid DNA by conventional method followed by sequencing to confirm the said SOD gene.

[0060] In an embodiment of the present invention, the polyclonal antibodies were raised against the purified SOD and used for cDNA library screening synthesized from young leaf mRNA.

[0061] In another embodiment of the present invention, library was screened and positive cDNA clones were amplified by polymerase chain reaction (hereinafter called as PCR) and two PCR products were obtained. These were sequenced and approximately 85% of the gene encoding SOD was obtained.

[0062] Further, in another embodiment of the present invention, the sequences of the said cDNA clones does not have the start and end codon and smaller by 21%.

[0063] In yet another embodiment of the present invention, primers were designed based on the sequences of positive cDNA clones and the rapid amplification of cDNA ends technique (hereinafter called as RACE) was employed to amplify the SOD full length gene.

[0064] In still another embodiment of the present invention, the said SOD gene is sequenced and analyzed comprising the sequences set forth in SEQ ID No. 2.

[0065] In still another embodiment of the present invention, the said full length SOD gene contains 856 nucleotide bases.

[0066] In still another embodiment of the present invention, the said full length SOD gene has entire coding sequence along with pre- and post-coding sequences.

[0067] In still another embodiment of the present invention, a set of primers are designed based on the full length SOD gene to amplify the superoxide dismutase (SOD) gene coding cDNA of SEQ ID No. 3.

[0068] In still another embodiment of the present invention, the said coding cDNA comprises 459 nucleotide bases.

[0069] In still another embodiment of the present invention, said SOD gene is ligated into a vector to yield a recombinant plasmid which upon transformation into a suitable *E. coli* host resulted into a clone.

[0070] In still another embodiment of the present invention, the said coding sequence of SOD gene of SEQ ID No. 3 corresponding to polynucleotides encoding Superoxide dismutase (SOD) enzyme.

[0071] Further, the present invention also provides an expression construct included sequences encoding a selectable marker and a terminator sequence.

[0072] In the present invention, leaves of *Potentilla* plant growing at Kunzum Pass (altitude 4517 m; 32° 24' N; 077° 38' E) in Lahaul and Spiti district of Himachal Pradesh in Western Himalaya of India were collected and stored in liquid nitrogen. We had earlier reported in our U.S. Pat. NO. 6,485,950 that the leaves of this plant has SOD that is autoclavable and functions at sub-zero temperature. Thus the gene encoding such a SOD was identified, isolated and cloned in *E.coli* by techniques well known and routinely practiced in the art. The present SOD gene was sequenced and analyzed for its sense orientation, comprising the sequences set forth in SEQ ID No;2. The term "sense" as used herein, refers to a substantial run of RNA bases having essentially the same bases as a specific RNA sequence (e.g., mRNA). The invention also embraced polynucleotides encoding the amino acid sequences set out in SEQ ID No. 1. The invention also provided host cells, comprising a polynucleotide of the invention in a manner that permits expression of the encoded SOD polypeptide. Suitable host cells for transformation with the SOD gene of the invention include bacterial cells e.g. *E. coli.* Polynucleotides of the invention may be introduced into the host cell as a part of a circular plasmid using the well known methods for introducing DNA into the host cell and routinely practiced in the art. Host cells of the invention are a valuable source for industrial scale production of recombinant SOD.

[0073] Polyclonal antibody, in the present invention refers to an antibody produced in the normal immune system in response to an antigen consists of a number of closely related, but not identical proteins).

[0074] Vector, in the present invention refers to the sequence of DNA capable of accepting foreign DNA and take the form of a circular plasmid DNA that shows resistance to a given antibiotic The gene sequence of the invention was compared with the SOD reported from other plants to figure out the uniqueness of the gene (FIG. **2**). Sequences unique to the polypeptides of the invention are recognizable through sequence comparison to other known polypeptides, and can be identified through use of alignment programs routinely utilized in the art, e.g., those made available in public sequence databases (FIG. **3**). This suggested that the sequence obtained were incomplete. SEQ ID No;3 however, shared at least 80%, at least 82%, at least 83%, at least 85%, at least 86% sequence homology with SOD genes reported from other plants. Percent sequence "homology" with respect to polynucleotides of the invention is defined herein as the percentage of nucleotide bases in the candidate sequence that are identical to the nucleotides in the SOD coding sequence after aligning the sequences, if necessary, to achieve the maximum percent sequence identity.

[0075] It is cumulative effect/combination of amino acids for the entire 100% amino acid composition that this property is observed. This entire composition provides this protein the effect that protein has this effect.

[0076] The following examples are given by way of illustration of the present invention and should not be construed to limit the scope of the present invention.

EXAMPLE-1

[0077] Raising Antibodies Against SOD in Rabbit

[0078] Polyclonal antibodies against purified protein were raised in one-year-old male rabbit (New Zealand type). Purified SOD protein (100 μg in 500 μl of potassium phosphate buffer; pH, 7.0) was emulsified in 1 ml of Freund's complete adjuvant and administered intramuscularly using disposable syringe. Complete Freund's adjuvant was obtained from Bangalore Genei, India that contained paraffin oil, mannide monooleate as an emulsifier and heat killed *Mycobacterium tuberculosis.* After 7th days of primary injection, a booster dose (1 ml, containing 60 μg of purified protein emulsified in 1 ml of incomplete adjuvant) was administered. Adjuvant (500 μl) was thoroughly emulsified with the purified enzyme (500 μl: 100 μg) to obtain a stable antigen-adjuvant emulsion by rapidly withdrawing and expelling the antigen-adjuvant mix using a 22 gauge needle fitted to a sterile syringe. Complete emulsification was tested by placing a drop of the mixture onto a still surface of distilled water. The intactness of the droplet assures complete mixing. Antigen-adjuvant mixture (800 μl) was injected in thigh muscles of rabbit using a 22 gauge needle. Blood was collected from heart of the rabbit and allowed to clot for 2 hours at room temperature. After overnight storage at 4° C., the edges of the clot were rimmed using a Pasture pipette and centrifuged at 150×g for 5 min. Supernatant was collected and centrifuged for 15 min at 350×g to remove cell debris. Sodium azide was added to a concentration of 0.025% and the serum was stored at 4° C. After second booster dose, a small amount of blood was collected to test for the presence of the antibody using Ouchterlony Double Diffussion (hereinafter known as ODD) as described by Kanematsu, S. and Asada, K.(1990) Plant Cell Physiol. 31: 99-112. Thus, in a 85 mm petri plate, 1.5% agar prepared in 0.15 M NaCl, 20 mM potassium phosphate of pH 7.0 and 0.02% sodium azide was poured to a thickness of 3 mm. Antigen (20 μl containing 4 μg of protein) and antibody were loaded into the 3 mm diameter well cut with the help of cork-borer. Petri plate was covered and kept in a humid environment for 16-24 hour at 37° C. and examined for line of immune precipitation.

EXAMPLE-2

[0079] RNA Isolation, Quantification of RNA, Gel-Electrophoresis and Purification of Poly A^+ mRNA from Total RNA

[0080] Ribonucleic acid (hereinafter known as, RNA) from young leaf tissue of *Potentilla* was isolated using the modified guanidine hydrochloride procedure (Lal. L., Sahoo. R., Gupta. R. K., Sharma. P. and kumar. S. Plant Molecular Biology Reporter 19: 181a-181f.). Leaf tissue (500 mg) was ground in liquid nitrogen to fine powder. Powder was transferred into a new mortar containing 5 ml of the GH buffer (8M guanidine hydrochloride, 20 mM EDTA, 20 mM MES, 100 mM βME) and was ground further. Resulting homogenate was transferred to an oak-ridge tube containing equal volume of phenol:chloroform:isoamylalcohal (25:24:1). Phases were emulsified by vortexing and separated by centrifugation at 10,000 rpm for 20 min (7° C.). Upper aqueous phase was transferred to a fresh oak-ridge tube and extracted with the equal volume of chloroform:isoamylalcohal (24:1). Resulting upper aqueous phase was transferred to a corex tube and RNA was precipitated by adding 0.2 volume of 1 M acetic acid and 0.7 volume of chilled ethanol. The tubes were kept at −72° C. for 3 h. Precipitate was pelleted by centrifugation at 10,000 rpm for 10 min at 4° C. Pellet was washed thrice using 5 ml of 3 M sodium acetate (pH 5.2) followed by final washing

with 70% chilled ethanol. Pellet was dried and dissolved in minimum volume of DEPC-treated autoclaved water. RNA was quantified by measuring absorbance at 260 nm and the purity was monitored by calculating the ratio of absorbance measured at 260 and 280 nm. A value >1.8 at 260/280 nm was considered ideal for the purity of RNA used in the present investigation. The formula used to calculate RNA concentration and yield was as follows:

Concentration of RNA (μg/ml)=A_{260} (absorbance at 260 nm)×40×dilution factor

Total yield (μg)=concentration×volume of stock RNA sample

[0081] To check the integrity of RNA, 5-6 μg of RNA in 4.5 μl of DEPC treated autoclaved water was diluted with 15.5 μl of M1 solution (2 μl of 5× MOPS buffer, 3.5 μl of formaldehyde, and 10 μl of formamide [5× MOPS buffer: 300 mM sodium acetate, 10 mM MOPS (3-{N-morpholino] propanesulfonic acid}, 0.5 mM ethylene diamine tetra-acetic acid (EDTA)] and incubated for 15 minutes at 65° C. RNA was loaded onto 1.0% formaldehyde agarose-gel after adding 2 μl of formaldehyde-gel loading buffer [50% glycerol, 1 mM EDTA (pH, 8.0), 0.25% bromophenol blue, 0.25% xylene cyanol FF], and electrophoresed at 72 volts in 1× MOPS buffer (60 mM sodium acetate, 2 mM MOPS, 0.1 mM EDTA), (Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, N.Y.).

[0082] Poly-A mRNA was purified from the total RNA using $dC_{10}T_{30}$ oligonucleotides attached covalently to polystyrene-latex particles (Oligotex™, Qiagen Inc). Oligotex selectively binds mRNA with poly-A tail to allow purification leaving all other RNA species that lack poly-A tail. 1 mg of total RNA was used as the starting material for the isolation of the mRNA and manufacturer's instructions were followed during the procedure.

EXAMPLE-3

[0083] Construction of a Directional Complementary DNA Library (Hereinafter Referred to cDNA Library)

[0084] Poly-A^+ mRNA was used to synthesize cDNA using TimeSaver™ cDNA synthesis kit (Amersham Pharmacia Biotech. USA). First strand was synthesized using MMLV-reverse transcriptase in the presence of a bifunctional primer [5'd(AAC TGG AAG AAT TCG CGG CCG CAG GAA T_{18})p 3'] having an oligo (dT_{18}) tract at the 3'-end of a restriction site for Not I. Second strand synthesis is initiated by DNA polymerase I after RNase H has nicked the RNA strand of the RNA:cDNA hybrid. The cDNA produced is extracted with phenol/chloroform and purified on a Sepharose CL-4B spun column. An Eco RI adaptor (5'-d[AATTCGGCACGAGG]-3', [GCCGTGCTCC]p-5') is ligated to other end of the cDNA. cDNA was digested with Not I to release site on oligo (dT_{18}-Not I) primer. cDNA's with Eco RI and Not I overhangs were phosphorylated to disallow self-ligation but ligation to the dephosphorylated vector.

[0085] Bacteriophage λ vector (λ ExCell Not I/Eco RI/CIP) was selected for cloning of cDNA's with Eco RI and Not I overhang generated as above. λ ExCell is derived from a λ Charon vector engineered to contain an internal, linearized copy of pExCell. Following the construction and screening using the lawn cells (*E. coli* strain NM522), the bacteriophage containing the clone of interest were used to infect a special *E. coli* strain (NP66) that enables the in vivo release of pExCell, a circular, autonomously replicating pUC-based phagemid. In vivo excision of pExCell is accomplished by site-specific recombination between attL and attR sites that flank the phagemid within the λ ExCell DNA. NP66 carries the accessory proteins require for excision under the control of a thermo-inducible promoter. In vivo excision is accomplished by infection of NP66 with λ ExCell followed by growth at 39° C., which enables the expression of these accessory proteins.

[0086] The ligated vector and the cDNA fragments were packaged in an in vitro packaging system (Ready to Go Lambda packaging kit, Amersham Pharmacia Biotech. USA). In vitro packaging system for lambda DNA uses single lysogen, which codes for all necessary packaging proteins. The extract was prepared from *E.coli* lysogen in which the prophage carries a cos mutation. The cos mutation is a deletion in the cos site which prevents the endogenous prophage from being packaged; the exogenous recombinant DNA is, however, efficiently packaged. Packaging extracts also lack Eco K and other DNA restriction systems that recognize methylated DNA, which results in the efficient packaging of methylated and unmethylated cDNA.

EXAMPLE-4

[0087] Library Screening and Identification, Amplification and Purification of Positive Phage

[0088] Library was screened using polyclonal antibodies raised against purified SOD as probe. Immobilized antibodies were detected using chemiluminescence based detection method (ECL™ western blotting analysis system, Amersham Inc.). The library was plated by making the serial dilutions of packaging reaction in SM buffer (100 mM NaCl, 8 mM $MgSO_4$, 50 mM Tris-HCl and 0.01% gelatin). Autoclaved and dried nitrocellulose filter membranes fitting to the size of petri plate (82 mm) were used. Membranes were soaked in 10 mM isopropyl β-D-thiogalactopyranoside (hereinafter referred to IPTG) for 5 min, air-dried and used for screening. After 6 h incubation of the plated library or as the plaques started appearing, the plates were overlaid with IPTG-soaked nitrocellulose filters. The filters were overlaid by gently holding filters with blunt ended forceps at opposite edges and centering filter over plate, without trapping any air bubble. Filter was not moved once contact is made with the plate. The plates-filters (inverted) were incubated for another 4 h at 37° C. After incubation, the plates were marked with 18-gauge needle by puncturing asymmetrically for future alignment. Filters were removed from plates with protein side up. Positive plaques were selected using the correct orientation of the developed X-ray, filter and the plate. After marking plagues were cored out and placed in 300 μl of SM buffer for incubation at room temperature. After 2 h, it was centrifuged at 13,000×g for 10 min. The supernatant was collected in a fresh sterile tube followed by addition of 30 μl chloroform. The amplified phage was replated for secondary and tertiary screening. After tertiary screening the positive plaques were cored for in vivo phagemid release. For primary screening 10^5 plaque forming unit (pfu) were taken and transferred to membrane. Membranes were hybridized with polyclonal antibody and developed as ECL instruction. Three strong positive clones were obtained and further taken for secondary screening which

gave 70% positive signal. A few clones were taken for tertiary screening and this time all the clones gave 100% positive signal after tertiary screening. All the positive plaques were used to release the vector pExCell containing the cloned fragment.

EXAMPLE-5

[0089] In Vivo Release of Phagemid pExcell from Selected Clones

[0090] Host cells were prepared from released strain of *E.coli* NP66 in 2× YT medium (2× YT medium: 12 g trypton, 24 g yeast extract and 5 g glycerol in 1 litre of final volume in distilled/deionized water) containing 50 μg/ml spectinomycin, 30 μg/ml of chloramphenicol and 0.2% maltose. The culture was grown overnight at 32° C. 5ml of 2× YT containing 50 μg/ml spectinomycin, 30 μg/ml of chloramphenicol and 0.2% maltose was incubated with 50 μl of the overnight culture. This culture was grown at 32° C. with shaking to an A_{600} of 0.5-0.8. and cells were harvested by centrifugation at 3000×g. The pellet was re-suspended NZCYM broth (NZCYM broth: 10 g casein hydrolysate, 5 g yeast extract, 5 g NaCl, 1 g casamino acid and 2 g $MgSO_4.7H_2O$ in 1 litre of final volume in distilled/deionized water) containing 50 μg/ml spectinomycin to a final A_{600} of 2.0. Cells were used within 1 h. To release the pExCell, 100 μl of the prepared NP66 cells were placed in a 15 ml sterile glass tube and incubated at 39° C. for 20 min to allow for expression of the His proteins required for site-specific recombination between attL and attR sites. 100 μl of the phage SM solution was added from to the cells and incubated at 39° C. for an additional 20 min. To this NP66/phage mixture, 200 μl of 1 M sodium citrate was added to terminate the infection of NP66 with λ ExCell and 5 ml of pre-warmed (32° C.) 2× YT broth containing 50 μg/ml spectinomycin was added. The culture was incubated at 32° C. with moderate shaking for 1.5 h to yield 'released culture'. To prepare overnight cultures for subsequent isolation of pExCell DNA, 50 μl of the released culture was incubated at 37° C. in 5 ml of LB medium (LB medium: 10 g trypton, 5 g yeast extract, 10 g sodium chloride in 1 litre of final volume in distilled/deionized water) containing 100 μg/ml ampicillin.

EXAMPLE-6

[0091] Analysis and Sequencing of Cloned cDNA

[0092] The cultures were streaked and the colonies were randomly picked up using a pipette tip. The colony was suspended in 50 μl of lysis buffer (colony lysis buffer: TE (Tris-Cl 10 mM, 1 mM EDTA, pH 8.0) with 0.1% tween 20), boiled for 10 min in a water bath followed by snap cooling on ice. Plasmid released in the colony lysate was amplified using 0.2 μM of each 'forward'(5'-GTTGTAAAACGACGGCCAGT-3') and 'reverse' (5'-CACAGGAAACAGCTATGACC-3') flanking primer, 20 μM of dNTPs and 1 Units of *Thermus aquaticus* (hereinafter referred to Taq) DNA polymerase (purchased from M/S. Qiagen, Germany) in 1× PCR buffer (20 mM Tris-Cl (pH, 8.4), 50 mM KCl, 1.5 mM $MgCl_2$). In the present invention, dNTPs refere to deoxy nucleoside triphosphate which comprises of deoxyadenosine triphosphate (hereinafter referred to dATP), deoxyguanosine triphosphate (hereinafter referred to dGTP), deoxycytidine triphosphate (hereinafter referred to dCTP) and deoxythymidine triphosphate (hereinafter referred to dTTP). Thermocycler program consisted of 30 cycles of 94° C. for 40 sec, 52° C. for 1 min and 72° C. for 2 min. This was followed by a 5 min extension at 72° C. Amplified products were run on 1.2% agarose gel in 1× TAE buffer (TAE buffer: 0.04 M Tris-acetate, 0.002 M EDTA, pH 8.5) containing ethidium bromide (final concentration of 0.5 μg/ml) and analyzed for correct size of insert by comparing with standard DNA molecular weight marker. Plasmids were isolated using QIAGEN plasmid mini kit (Cat#12125). These were quantified, checked on 1% agarose gel and sequencing was performed using the BigDye terminator (version 3.1) cycle sequencing mix (Applied Biosystems, USA) on automated DNA sequencer (ABI Prism 310, Genetic Analyzer, Applied Biosystems, USA). Protocols were followed essentially as described by respective manufacturers. Sequencing primers used were 'forward' 5'-

INFORMATION FOR SEQ ID NO:4
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 365 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:4

5'CAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACATTTCTGGCCTC

AAGCCTGGGCTTCATGGTTTCCATGTTCATGCTCTTGGGGACACAACCAA

TGGTTGCATGTCAACTGGACCACATTTCAATCCTGCTGGCAAAGAGCATG

GGTCTCCTGAAGATGAGACTCGTCATGCTGGTGATCTTGGAAATATCACT

GTTGGGGATGACGGAACTGCTTGCTTCACAATTGTTGACAAACAGATTCC

TCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGTTGTCCATGCAG

ATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCAAATCCACTGGA

AATGCTGGTGGCAGGAT 3'

EXAMPLE-7

[0093] Sequence mentioned in example 6 was searched for homology in the gene databases available at URL www.ncbi.nlm.nih.gov. using Basic Local Alignment Search Tool (hereinafter called as BLAST). It was clear from the results that the sequence had a homology between 80-90% with the SOD sequences submitted in the databases.

EXAMPLE-8

[0094] Cloning of Full Length Gene Using Rapid Amplification of cDNA Ends (Hereinafter Referred to RACE)

[0095] Rapid amplification of cDNA ends (RACE) was used to isolate full length SOD gene from *Potentilla atrosanguinea*. RACE amplifies DNA sequences from a messenger RNA template between a defined internal site and unknown sequences of either the 3' or 5' end (Frohman, M. A., Dush. M. K. and Martin, G. R. (1988) Proc. Natl. Acad. Sci. USA 85: 8998-9002; U.S. Pat. Nos. 5,962,271 and 5,962,272). A set of gene specific primers were used to

generate 5' and 3' ends of the gene separately. The partial cDNA sequence (SEQ ID No; 1) was used to design two sets of primers. Primers were designed such that the amplified 5' and 3' ends overlap each other over a small stretch of nucleotides. For 5' RACE, a gene specific primer (hereinafter referred to GSP1),5'-CCAGTGGATTTGCTAAGCTCATGTCCA-3' for primary PCR and one nested gene specific primer (hereinafter referred to NES1), 5'-GTCATCAGGGTCTGCATGGACAACAAC-3' for secondary PCR (RACE). It has been used 1 μl of 10 μM nested primers stock for secondary PCR. For 3' RACE a gene specific primer (hereinafter referred to GSP2), 5'-ATGGTTGCATGTCAACTGGACCACATT-3' for primary PCR and one nested primer (hereinafter referred to NES2), 5'-TTGCATGTCAACTGGACCACATTTCAA-3' were designed. Primers were designed such that the amplified 5' and 3' ends overlap each other over a small stretch of nucleotides.

[0096] The cDNA for 5'-RACE was synthesized using a modified lock-docking oligo(dT) primer and SMART II A oligo (dT) primer. The modified oligo (dT) primer, termed the 5'-RACE CDS Primer (5'-CDS) has two degenerate nucleotide positions at the 3' end.

[0097] 1 μg of total RNA was reverse transcribed in separate reactions to yield 5' and 3' RACE ready cDNA using an enzyme known as reverse transcriptase. For 5' cDNA synthesis, the reaction was carried out using 1 μM of 5'-CDS primer in a reaction mixture containing RNA and 1 μM SMART II oligo (dT) primer. The 3'-RACE cDNA is synthesized using a traditional reverse transcription procedure, but with a special oligo (dT) primer. This 3'-RACE CDS Primer A (3'-CDS) primer includes the lock-docking nucleotide positions as in the 5'-CDS and also has a portion of the smart sequence at its 5' end. Sterile H_2O was added to a final volume of 5 μl for each reaction, mixed and centrifuged. The reaction mix was incubated at 70° C. for 2 min and cooled on ice for 2 min. First-strand buffer (50 mM Tris-Cl (pH, 8.3), 75 mM KCl and 6 mM $MgCl_2$), 1 mM dNTPs, 2mM DTT and reverse transcriptase were added to each reaction and incubated at 42° C. for 1.5 hr in an air incubator. Diluted the first-strand reaction product with 100 μl of Tricine-EDTA buffer (10 mM Tricine-KOH (pH 8.5), 1.0 mM EDTA) and heated tubes at 72° C. for 7 min. (Reverse transcription system was a component of SMART RACE cDNA amplification kit from BD Biosciences, USA).

[0098] Sequences of Primers Used for RACE Were as Follows (Purchased from BD Biosciences, USA as a Part of RACE Kit).

Primer	Primer Sequence
SMART II A Oligonucleotide	5'-AAGCAGTGGTATCAACGCAGAGTACGCGGG-3'
3'-RACE CDS Primer A (3'-CDS)	5'-AAGCAGTGGTATCAACGCAGAGTAC$(T)_{30}N_{-1}N$-3'
5'-RACE CDS Primer (5'-CDS)	5'-$(T)_{25}N_{-1}N$-3'
10X Universel Primer Mix A (UPM)	Long: 5'-TAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3'
	Short: 5'-CTAATACGACTCACTATAGGGC-3'
Nested Universal Primer A (NUP)	5'-AAGCAGTGGTATCAACGCAGAGT-3'

-continued

[0099] 5' and 3' RACE cDNA were amplified using 0.2 μM GSP1, GSP2 primer and 1× universal primer (UPM), 0.2 mM dNTP and 1× BD polymerase mix. Thermocycler program consisted of 30 cycles of 94° C. for 30 sec, 68° C. for 30 sec and 72° C. for 3 min. The reaction was up-scaled to 50 μl and after the completion of PCR, 45 μl of PCR sample was run on 1.2% agarose gel in TAE buffer containing ethidium bromide (final concentration of 0.5 μg/ml) Rest of the amplified product was stored at −20° C. for secondary PCR if needed. Amplicons were cut from the gel and DNA was eluted from the gel using QIAEX II gel extraction kit from M/S Qiagen, Germany following the manufacturer's instructions. The purified DNA was cloned in pGEM-T easy vector (Promega, USA), plasmids were isolated using the Qiagen plasmid mini-isolation kit, and sequencing was performed using the BigDye terminator (version 3.1) cycle sequencing mix (Applied Biosystems, USA) on an automated DNA sequencer (ABI Prism 310, Genetic Analyzer, Applied Biosystems). Protocols were followed essentially as described by respective manufacturers. The RACE products were analyzed by BLAST.

(3) INFORMATION FOR SEQ ID NO:2
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 856 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA

(iii) SEQUENCE DESCRIPTION: SEQ ID NO:2

5'ACGGGGGGGGGACTGAAATAAATAGAGAGGGTCATAGTCACATTTGCA

TTTAGGTATCTGATTCCATTCACAAACCTCCAACTCCCACCTCTCTCTCT

ATTTCTCTTCATCTTCATCATCTTAGGGTGCACTGAGATCACTTTGAAAC

ATGGCAAAGGGCGTTGCTGTACTTAGCTCCAGTGAGGGTGTTGCTGGAAC

TATCCTCTTTACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAAACA

TTTCTGGCCTCAAGCCTGGGCTTCATGGTTTCCATGTTCATGCTCTTGGG

GACACAACCAATGGTTGCATGTCAACTGGACCACATTTCAATCCTGCTGG

CAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCATGCTGGTGATCTTG

GAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTGTTGAC

AAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGTTGT

TGTCCATGCAGATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTAGCA

-continued

AATCCACTGGAAATGCTGGTGGCAGGATAGCTTGTGGTATTATTGGCCTT

CAAGGATGAACTGGACCAGGGAGCGAAACACAGGCATCTTGTTGAATTAA

AACTTGAGATATTAGCGAACTCTTCGGAATTGAGTATTGAAACAAGGAAT

ACATTTGTCATTACCAATACGTTTGGCTTAGACCTGTATTCTGTATCTCA

ATAGTTTTCTGTGTGGTTGTTTGACAGTTATTTGTGCTCAGGCTATTTCA

AAGGGATAAACACAGTAACTTTCTTGCTTTGACAAAAAAAAAAAAAAAAA

AAAAAAAA 3'

EXAMPLE-9

[0100] Amplification of Coding Sequence (Hereinafter Known as CDS) of SOD and Cloning into an Expression Vector

[0101] CDS of SOD was amplified by PCR using the forward primer 5'-ATGGCAAAGGGCGTTGCTGTACTT-3' and reverse primer 5'-TCATCCTTGAAGGC-CAATAATACCA-3' designed from start codon and stop codon. The amplified product was cloned into pQE 30 expression vector and transformed into competent *E. coli* cells. The plasmid was isolated using the standard plasmid isolation protocol (Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Plainview, N.Y.) and sequencing was performed using the BigDye terminator (version 3.1) cycle sequencing mix (Applied Biosystems, USA) on an automated DNA sequencer (ABI Prism 310, Genetic Analyzer, Applied Biosystems) to confirm cloning of insert. Protocols were followed essentially as described by the manufacturer.

(4) INFORMATION FOR SEQ ID NO: 3

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 459 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: cDNA

(iii) SEQUENCE DESCRIPTION: SEQ ID NO: 3

5' ATGGCAAAGGGCGTTGCTGTACTTAGCTCCAGTGAGGGTGTTGCTGG

AACTATCCTCTTTACCCAAGAGGGAGATGGCCCAACTACTGTGACCGGAA

ACATTTCTGGCCTCAAGCCTGGGCTTCATGGTTTCCATGTTCATGCTCTT

GGGGACACAACCAATGGTTGCATGTCAACTGGACCACATTTCAATCCTGC

TGGCAAAGAGCATGGGTCTCCTGAAGATGAGACTCGTCATGCTGGTGATC

TTGGAAATATCACTGTTGGGGATGACGGAACTGCTTGCTTCACAATTGTT

GACAAACAGATTCCTCTCACTGGACCACACTCTATCATTGGTAGGGCTGT

TGTTGTCCATGCAGATCCTGATGACCTTGGCAAGGGTGGACATGAGCTTA

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GCAAATCCACTGGAAATGCTGGTGGCAGGATAGCTTGTGGTATTATTGGC

CTTCAAGGATGA 3'

(5) INFORMATION FOR Pro SEQ ID NO: 1

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 152 amino acids

(B) TYPE: amino acid

(ii) MOLECULE TYPE: polypeptide

(iii) SEQUENCE DESCRIPTION: Pro SEQ ID NO: 1

MAKGVAVLSSSEGVAGTILFTQEGDGPTTVTGNISGLKPGLHGFHVHALG

DTTNGCMSTGPHFNPAGKEHGSPEDETRHAGDLGNITVGDDGTACFTIVD

KQIPLTGPHSIIGRAVVVHADPDDLGKGGHELSKSTGNAGGRIACGIIGL

QG

EXAMPLE-10

[0102] Induction and Purification of Expressed Protein

[0103] *E. coli* containing SOD gene from *Potentilla* was grown at 37° C. in 100 ml of LB medium with 100 μg ml^{-1} and 25 μg ml^{-1} kanamycin. When that culture had grown to an absorbance of 0.6 at 600 nm, IPTG was added to a final concentration of 1 mM. $CuSO_4$ and $ZnSO_4$ were added to a final concentration of 100 ppm and 2 ppm, respectively. After inducing the expression of the SOD protein for 5 h at 37° C., cells were harvested, washed and resuspended in 4 ml of lysis buffer (50 mM NaH_2PO_4 buffer, pH 8.0, containing 300 mM NaCl and 10 mM imidazole). The cell suspension was sonicated, and the lysate was cleared by centrifugation at 12000 g and 4° C. for 20 min. The supernatant was then poured into the column loaded with nickel-nitrilotriacitic acid (Ni-NTA) agarose, washed with wash buffer (50 mM NaH_2PO_4 buffer, pH 8.0, containing 300 mM NaCl and 20 mM imidazole), and SOD protein was eluted with elution buffer (50 mM NaH_2PO_4 buffer, pH 8.0, containing 300 mM NaCl and 250 mM imidazole). The purified SOD was evaluated by 10% SDS-PAGE using silver staining to visualize the protein (FIG. **4**A).

[0104] The protein estimation was performed before and after autoclaving purified SOD that shows ±25% loss of protein. Since 50 μl of protein sample was used for assaying SOD activity, the loss in protein was calculated while calculating the enzyme activity. Reaction medium contained 0.05 M potassium phosphate buffer (pH, 7.0), 5.7×10^{-5} M nitroblue tetrazolium (hereinafter referred to NBT), 9.9×10^{-3} M methionine, 1.17×10^{-6} M riboflavin and 0.025% Triton X-100 in a total volume of 3.0 ml. Reaction (performed in a 30 ml glass vial) was initiated by illuminating the reaction with light intensity of 1000 μ Einstein/m^2/second using a fiber optic light source (Nikon). The reaction was terminated after 2 min and the absorbance was read at 560 nm.

[0105] A control reaction was always performed wherein all the steps and components were exactly the same as described above except that purified enzyme was replaced with equal volume of homogenizing buffer. Activity of SOD is expressed as per cent inhibition in color development as compared to the control reaction (higher the inhibition, higher the SOD activity). Activity data was shown in FIG. **1**.

EXAMPLE-11

[0106] SOD Activity at Different Temperatures in Purified SOD

[0107] The purified SOD enzyme was assayed at temperatures ranging between −10 to 80 ° C. in the buffer composition as described in Example 10 except that 50% glycerol was added in the reaction mixture to avoid freezing at low temperature. A glass beaker of 100 ml capacity was filled with either alcohol (for working at temperatures of −10, −5, 0° C.) or distilled water (for working at rest of the temperatures) was used to maintain the temperature of the reaction medium while assaying SOD. Reaction medium along with the enzyme was pre-equilibrated at desired temperature to avoid time lag in attaining the required temperature. As can be seen from FIG. **1** that the enzyme showed highest activity (87.5% inhibition) at 0° C. The enzyme was functional even up to −10° C. (82% inhibition). The enzyme is expected at temperature lower than −10° C.

[0108] Control reactions, as mentioned in Example 10, were always performed at all the temperatures.

EXAMPLE-12

[0109] Localization of SOD by Activity Staining of Native Gel

[0110] The purified SOD was localized on 10% polyacrylamide gel by activity staining as described by Beauchamp and Fridovich (Anal. Biochem. 1971; 44: 246-287). After electrophoresis, the gel was rinsed with distilled water followed by 30 min incubation in 50 ml phosphate buffer (50 mM; pH 7.0) containing 2.5 mM NBT in dark at room temperature. Gel was then immersed in $1.17\times10^{-}6$ M riboflavin for 20 min, followed by exposure to light source (Nikon). Light exposure led to photogeneration of O_2^-, which converts NBT into insoluble purple colored formazan. The purple color was developed throughout the gel except for the location where SOD was localized as shown in FIG. **4**B.

ADVANTAGES

[0111] The main advantages of the present invention are:

[0112] 1. SOD gene has been cloned from *Potentilla* that is autoclavable and functions at sub-zero temperature.

[0113] 2. SOD gene that is isolated from *Potentilla* has been made to express in *E. coli.*

[0114] 3. SOD gene from *Potentilla* that is made to express in *E.coli* leading to synthesis of SOD protein that is autoclavable.

[0115] 4. SOD gene from *Potentilla* that is made to express in *E.coli* leading to synthesis of SOD protein that is autoclavable, also functions at sub-zero temperature.

SEQUENCE LISTING

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<213> ORGANISM: Potentilla atrosanguinea

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Asn Ile Ser Gly Leu Lys Pro Gly Leu His Gly Phe His Val His Ala
         35                  40                  45

Leu Gly Asp Thr Thr Asn Gly Cys Met Ser Thr Gly Pro His Phe Asn
     50                  55                  60

Pro Ala Gly Lys Glu His Gly Ser Pro Glu Asp Glu Thr Arg His Ala
 65                  70                  75                  80

Gly Asp Leu Gly Asn Ile Thr Val Gly Asp Asp Gly Thr Ala Cys Phe
                 85                  90                  95

Thr Ile Val Asp Lys Gln Ile Pro Leu Thr Gly Pro His Ser Ile Ile
            100                 105                 110

Gly Arg Ala Val Val Val His Ala Asp Pro Asp Asp Leu Gly Lys Gly
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115 120 125

Gly His Glu Leu Ser Lys Ser Thr Gly Asn Ala Gly Gly Arg Ile Ala
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Cys Gly Ile Ile Gly Leu Gln Gly
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ttagggtgca ctgagatcac tttgaaacat ggcaaagggc gttgctgtac ttagctccag 180

tgagggtgtt gctggaacta tcctctttac ccaagaggga gatggcccaa ctactgtgac 240

cggaaacatt tctggcctca agcctgggct tcatggtttc catgttcatg ctcttgggga 300

cacaaccaat ggttgcatgt caactggacc acatttcaat cctgctggca aagagcatgg 360

gtctcctgaa gatgagactc gtcatgctgg tgatcttgga aatatcactg ttggggatga 420

cggaactgct tgcttcacaa ttgttgacaa acagattcct ctcactggac cacactctat 480

cattggtagg gctgttgttg tccatgcaga tcctgatgac cttggcaagg gtggacatga 540

gcttagcaaa tccactggaa atgctggtgg caggatagct tgtggtatta ttggccttca 600

aggatgaact ggaccaggga gcgaaacaca ggcatcttgt tgaattaaaa cttgagatat 660

tagcgaactc ttcggaattg agtattgaaa caaggaatac atttgtcatt accaatacgt 720

ttggcttaga cctgtattct gtatctcaat agttttctgt gtggttgttt gacagttatt 780

tgtgctcagg ctatttcaaa gggataaaca cagtaacttt cttgctttga caaaaaaaaa 840

aaaaaaaaaa aaaaaa 856

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<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 3

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cttcatggtt tccatgttca tgctcttggg gacacaacca atggttgcat gtcaactgga 180

ccacatttca atcctgctgg caaagagcat gggtctcctg aagatgagac tcgtcatgct 240

ggtgatcttg gaaatatcac tgttggggat gacggaactg cttgcttcac aattgttgac 300

aaacagattc ctctcactgg accacactct atcattggta gggctgttgt tgtccatgca 360

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catttcaatc ctgctggcaa agagcatggg tctcctgaag atgagactcg tcatgctggt 180

gatcttggaa atatcactgt tggggatgac ggaactgctt gcttcacaat tgttgacaaa 240

cagattcctc tcactggacc acactctatc attggtaggg ctgttgttgt ccatgcagat 300

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<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide
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<222> LOCATION: (27)..(28)
<223> OTHER INFORMATION: a, c, g, t, unknown or other

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<223> OTHER INFORMATION: a, c, g, t, unknown or other

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<400> SEQUENCE: 21

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<210> SEQ ID NO 22
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<212> TYPE: DNA
<213> ORGANISM: Malus sp.

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cttcatggtt tccatgtcca tgctcttgga gacacaacaa acggttgcat gtcaactggg 180

ccacacttca atcctgctgg aaaagagcat ggtgcccctg aagatgagct tcgccatgct 240

ggcgatcttg gaaacatcac tgctggggac gatggaactg caaccttcac gattgttgac 300

aagcagattc ctctcgctgg accacactct atcattggta gggcggttgt tgtccacgca 360

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cttcatggct tccacgtcca tgcccttgga gacaccacaa atggctgcat gtcaactggg 180

ccgcatttta atcctgtagg caaggagcat ggtgcccctg aggatgagaa tcgtcatgct 240

ggtgatctgg gaaatgtcac tgttggtgat gatggcactg ctgctttcac aatcattgac 300

aaacagattc ctcttactgg accacattcc attattggtt gggctgttgt tgttcatgga 360

gatcctgatg atcttggcaa gggaggacat gaactcagca aaaccactgg taatgctggc 420

ggcagagtag catgcggtat tattggtctg caaggttga 459

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ctccacggct tccatatcca tgccttggga gacaccacaa acggttgcat ttcaactgga 180

ccacatttca atcctaatgg gaaggaacat ggtgcccctg aggatgagac tagacatgct 240

ggtgatcttg gaaatatcaa tgttggtgat gatggaactg taagcttcac cattactgac 300

aaccatatcc ctctcactgg aacaaactcc atcataggaa gggctgttgt tgtccatgcc 360

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<213> ORGANISM: Arabidopsis sp.

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cttcatggtt tccatgtcca tgctcttggt gacaccacta acggttgcat gtctactggt 180

ccacatttca accccgatgg taaaacacac ggtgcccctg aggatgctaa tcgacatgct 240

ggtgatctag gaaacatcac tgttggagat gatggaactg ccaccttcac aatcactgat 300

tgccagattc ctcttactgg accaaactct attgttggta gggctgttgt tgtccatgca 360

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ctccatggat tccatgtgca cgcgctcggt gacaccacta atggctgcat gtcaactgga 180

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ccacacttca atcctactgg gaaggaacat ggggcaccac aagatgagaa ccgccatgcc 240

ggtgatcttg gaaatataac agctggagca gatggtgttg ctaatgtcaa tgtctctgac 300

agccagatcc cccttactgg agcacactcc atcattggcc gagctgttgt tgtccatgct 360

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<213> ORGANISM: Malus sp.

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35 40 45

Leu Gly Asp Thr Thr Asn Gly Cys Met Ser Thr Gly Pro His Phe Asn
50 55 60

Pro Ala Gly Lys Glu His Gly Ala Pro Glu Asp Glu Leu Arg His Ala
65 70 75 80

Gly Asp Leu Gly Asn Ile Thr Ala Gly Asp Asp Gly Thr Ala Thr Phe
85 90 95

Thr Ile Val Asp Lys Gln Ile Pro Leu Ala Gly Pro His Ser Ile Ile
100 105 110

Gly Arg Ala Val Val Val His Ala Asp Pro Asp Asp Leu Gly Lys Gly
115 120 125

Gly His Glu Leu Ser Lys Ser Thr Gly Asn Ala Gly Gly Arg Val Ala
130 135 140

Cys Gly Ile Ile Gly Leu Gln Gly
145 150

<210> SEQ ID NO 30
<211> LENGTH: 152
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis sp.

<400> SEQUENCE: 30

Met Ala Lys Gly Val Ala Val Leu Asn Ser Ser Glu Gly Val Thr Gly
1 5 10 15

Thr Ile Phe Phe Thr Gln Glu Gly Asp Gly Val Thr Thr Val Ser Gly
20 25 30

Thr Val Ser Gly Leu Lys Pro Gly Leu His Gly Phe His Val His Ala
35 40 45

Leu Gly Asp Thr Thr Asn Gly Cys Met Ser Thr Gly Pro His Phe Asn
50 55 60

Pro Asp Gly Lys Thr His Gly Ala Pro Glu Asp Ala Asn Arg His Ala
65 70 75 80

Gly Asp Leu Gly Asn Ile Thr Val Gly Asp Asp Gly Thr Ala Thr Phe
85 90 95

Thr Ile Thr Asp Cys Gln Ile Pro Leu Thr Gly Pro Asn Ser Ile Val

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            100                 105                 110

Gly Arg Ala Val Val Val His Ala Asp Pro Asp Asp Leu Gly Lys Gly
        115                 120                 125

Gly His Glu Leu Ser Leu Ala Thr Gly Asn Ala Gly Gly Arg Val Ala
    130                 135                 140

Cys Gly Ile Ile Gly Leu Gln Gly
145                 150


<210> SEQ ID NO 31
<211> LENGTH: 152
<212> TYPE: PRT
<213> ORGANISM: Populus sp.

<400> SEQUENCE: 31

Met Val Lys Ala Val Ala Val Leu Asn Ser Ser Glu Gly Val Ser Gly
  1               5                  10                  15

Thr Ile Phe Phe Thr Gln Glu Gly Asp Gly Pro Thr Thr Val Thr Gly
             20                  25                  30

Asn Leu Ser Gly Leu Lys Pro Gly Leu His Gly Phe His Val His Ala
         35                  40                  45

Leu Gly Asp Thr Thr Asn Gly Cys Met Ser Thr Gly Pro His Phe Asn
     50                  55                  60

Pro Val Gly Lys Glu His Gly Ala Pro Glu Asp Glu Asn Arg His Ala
 65                  70                  75                  80

Gly Asp Leu Gly Asn Val Thr Val Gly Asp Asp Gly Thr Ala Ala Phe
                 85                  90                  95

Thr Ile Ile Asp Phe Gln Ile Pro Leu Thr Gly Pro His Ser Ile Ile
            100                 105                 110

Gly Arg Ala Val Val Val His Gly Asp Pro Asp Asp Leu Gly Lys Gly
        115                 120                 125

Gly His Glu Leu Ser Lys Thr Thr Gly Asn Ala Gly Gly Arg Val Ala
    130                 135                 140

Cys Gly Ile Ile Gly Leu Gln Gly
145                 150


<210> SEQ ID NO 32
<211> LENGTH: 152
<212> TYPE: PRT
<213> ORGANISM: Oryza sp.

<400> SEQUENCE: 32

Met Val Lys Ala Val Val Val Leu Gly Ser Ser Glu Ile Val Lys Gly
  1               5                  10                  15

Thr Ile His Phe Val Gln Glu Gly Asp Gly Pro Thr Thr Val Thr Gly
             20                  25                  30

Ser Val Ser Gly Leu Lys Pro Gly Leu His Gly Phe His Ile His Ala
         35                  40                  45

Leu Gly Asp Thr Thr Asn Gly Cys Met Ser Thr Gly Pro His Tyr Asn
     50                  55                  60

Pro Ala Gly Lys Glu His Gly Ala Pro Glu Asp Glu Thr Arg His Ala
 65                  70                  75                  80

Gly Asp Leu Gly Asn Val Thr Ala Gly Glu Asp Gly Val Ala Asn Ile
                 85                  90                  95

His Val Val Asp Ser Gln Ile Pro Leu Thr Gly Pro Asn Ser Ile Ile
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            100                 105                 110

Gly Arg Ala Val Val Val His Ala Asp Pro Asp Asp Leu Gly Lys Gly
        115                 120                 125

Gly His Glu Leu Ser Lys Thr Thr Gly Asn Ala Gly Gly Arg Val Ala
    130                 135                 140

Cys Gly Ile Ile Gly Leu Gln Gly
145                 150


<210> SEQ ID NO 33
<211> LENGTH: 152
<212> TYPE: PRT
<213> ORGANISM: Zea sp.

<400> SEQUENCE: 33

Met Val Lys Ala Val Ala Val Leu Gly Ser Ser Glu Gly Val Lys Gly
  1               5                  10                  15

Thr Ile Phe Phe Thr Gln Glu Gly Asp Gly Pro Thr Thr Val Thr Gly
             20                  25                  30

Ser Val Ser Gly Leu Lys Pro Gly Leu His Gly Phe His Val His Ala
         35                  40                  45

Leu Gly Asp Thr Thr Asn Gly Cys Met Ser Thr Gly Pro His Tyr Asn
     50                  55                  60

Pro Ala Ser Lys Glu His Gly Ala Pro Glu Asp Glu Asn Arg His Ala
 65                  70                  75                  80

Gly Asp Leu Gly Asn Val Thr Ala Gly Ala Asp Gly Val Ala Asn Ile
                 85                  90                  95

Asn Val Thr Asp Ser Gln Ile Pro Leu Thr Gly Pro Asn Ser Ile Ile
            100                 105                 110

Gly Arg Ala Val Val Val His Ala Asp Pro Asp Asp Leu Gly Lys Gly
        115                 120                 125

Gly His Glu Leu Ser Lys Ser Thr Gly Asn Ala Gly Gly Arg Val Ala
    130                 135                 140

Cys Gly Ile Ile Gly Leu Gln Gly
145                 150


<210> SEQ ID NO 34
<211> LENGTH: 152
<212> TYPE: PRT
<213> ORGANISM: Gossypium sp.

<400> SEQUENCE: 34

Met Val Lys Ala Val Ala Val Leu Gly Ser Asn Glu Gly Val Ser Gly
  1               5                  10                  15

Thr Val Phe Phe Ser Gln Glu Gly Asp Gly Pro Thr Thr Val Thr Gly
             20                  25                  30

Asn Leu Ser Gly Leu Lys Pro Gly Leu His Gly Phe His Val His Ala
         35                  40                  45

Leu Gly Asp Thr Thr Asn Gly Cys Met Ser Thr Gly Pro His Phe Asn
     50                  55                  60

Pro Ala Gly Lys Glu His Gly Ala Pro Glu Asp Glu Asn Arg His Ala
 65                  70                  75                  80

Gly Asp Leu Gly Asn Val Thr Val Gly Asp Asp Gly Cys Ala Ser Phe
                 85                  90                  95

Ser Ile Thr Asp Lys Gln Ile Pro Leu Thr Gly Pro Asn Ser Ile Ile
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            100                 105                 110

Gly Arg Ala Val Val Val His Ala Asp Pro Asp Asp Leu Gly Lys Gly
        115                 120                 125

Gly His Glu Leu Ser Lys Ser Thr Gly Asn Ala Gly Gly Arg Val Ala
    130                 135                 140

Cys Gly Ile Ile Gly Leu Gln Gly
145                 150


<210> SEQ ID NO 35
<211> LENGTH: 152
<212> TYPE: PRT
<213> ORGANISM: Pisum sp.

<400> SEQUENCE: 35

Met Val Lys Ala Val Ala Val Leu Ser Asn Ser Asn Glu Val Ser Gly
  1               5                  10                  15

Thr Ile Asn Phe Ser Gln Glu Gly Asn Gly Pro Thr Thr Val Thr Gly
             20                  25                  30

Thr Leu Ala Gly Leu Lys Pro Gly Leu His Gly Phe His Ile His Ala
         35                  40                  45

Leu Gly Asp Thr Thr Asn Gly Cys Ile Ser Thr Gly Pro His Phe Asn
     50                  55                  60

Pro Asn Gly Lys Glu His Gly Ala Pro Glu Asp Glu Thr Arg His Ala
 65                  70                  75                  80

Gly Asp Leu Gly Asn Ile Asn Val Gly Asp Asp Gly Thr Val Ser Phe
                 85                  90                  95

Thr Ile Thr Asp Asn His Ile Pro Leu Thr Gly Thr Asn Ser Ile Ile
            100                 105                 110

Gly Arg Ala Val Val Val His Ala Asp Pro Asp Asp Leu Gly Lys Gly
        115                 120                 125

Gly His Glu Leu Ser Lys Thr Thr Gly Asn Ala Gly Gly Arg Val Ala
    130                 135                 140

Cys Gly Ile Ile Gly Leu Gln Gly
145                 150


<210> SEQ ID NO 36
<211> LENGTH: 152
<212> TYPE: PRT
<213> ORGANISM: Glycine max

<400> SEQUENCE: 36

Met Val Lys Ala Val Ala Val Leu Gly Ser Ser Glu Gly Val Thr Gly
  1               5                  10                  15

Thr Ile Phe Phe Thr Gln Glu Gly Asn Gly Pro Thr Thr Val Thr Gly
             20                  25                  30

Ser Leu Ala Gly Leu Lys Pro Gly Leu His Gly Phe His Val His Ala
         35                  40                  45

Leu Gly Asp Thr Thr Asn Gly Cys Leu Ser Thr Gly Ala His Phe Asn
     50                  55                  60

Pro Asn Asn Asn Glu His Gly Ala Pro Glu Asp Glu Asn Arg His Ala
 65                  70                  75                  80

Gly Asp Leu Gly Asn Val Asn Val Gly Asp Asp Gly Thr Val Ser Phe
                 85                  90                  95

Ser Ile Thr Asp Ser Gln Ile Pro Leu Thr Gly Pro Asn Ser Ile Ile
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            100                 105                 110

Gly Arg Ala Val Val Val His Ala Asp Ser Asp Asp Leu Gly Lys Gly
        115                 120                 125

Gly His Glu Leu Ser Lys Thr Thr Gly Asn Ala Gly Gly Arg Val Ala
    130                 135                 140

Cys Gly Ile Ile Gly Leu Gln Gly
145                 150


<210> SEQ ID NO 37
<211> LENGTH: 394
<212> TYPE: DNA
<213> ORGANISM: Ipomoea batatas

<400> SEQUENCE: 37

tcttcagcca agaaggagat ggtccaacca cagtcactgg aaacgtttcg ggcctcaaac     60

ctggtcttca tggcttccat gtccatgccc taggtgacac aacaaatgga tgcatgtcta    120

ctggaccaca tttcaatcct gctggaaagg agcatggagc tcctggagac gataaccgcc    180

atgccggtga tcttggaaac atcacggttg gagaagatgg tactgcttca ttcaccatca    240

ctgacaagca gattccgctt actggagcaa attctgttat tggaagagct gttgttgttc    300

atggtgatcc cgatgatctt ggtaaaggtg gccatgagct cagcaaaagc actggaaatg    360

ctggcgggag ggttgcctgc ggtatcattg gcct                                394


<210> SEQ ID NO 38
<211> LENGTH: 394
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 38

tctttaccca agagggagat ggcccaacta ctgtgaccgg aaacatttct ggcctcaagc     60

ctgggcttca tggtttccat gttcatgctc ttggggacac aaccaatggt tgcatgtcaa    120

ctggaccaca tttcaatcct gctggcaaag agcatgggtc tcctgaagat gagactcgtc    180

atgctggtga tcttggaaat atcactgttg gggatgacgg aactgcttgc ttcacaattg    240

ttgacaaaca gattcctctc actggaccac actctatcat tggtagggct gttgttgtcc    300

atgcagatcc tgatgacctt ggcaagggtg gacatgagct tagcaaatcc actggaaatg    360

ctggtggcag gatagcttgt ggtattattg gcct                                394


<210> SEQ ID NO 39
<211> LENGTH: 459
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 39

atggcaaagg gcgttgctgt acttagctcc agtgagggtg ttgctggaac tatcctcttt     60

acccaagagg gagatggccc aactactgtg accggaaaca tttctggcct caagcctggg    120

cttcatggtt tccatgttca tgctcttggg gacacaacca atggttgcat gtcaactgga    180

ccacatttca atcctgctgg caaagagcat gggtctcctg aagatgagac tcgtcatgct    240

ggtgatcttg gaaatatcac tgttggggat gacggaactg cttgcttcac aattgttgac    300

aaacagattc ctctcactgg accacactct atcattggta gggctgttgt tgtccatgca    360

gatcctgatg accttggcaa gggtggacat gagcttagca aatccactgg aaatgctggt    420
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ggcaggatag cttgtggtat tattggcctt caaggatga 459

<210> SEQ ID NO 40
<211> LENGTH: 459
<212> TYPE: DNA
<213> ORGANISM: Fagus sylvatica

<400> SEQUENCE: 40

atggccaagg gtgtggctgt tcttagctcg aatgagggtg tttgtggcac tatctacttt 60

gcccaagaag gagatggccc aactacagta actggaaata tttctggcct taaacctgga 120

ctccatggct tccacgtgca tgctcttggg gacacaacaa atggttgcat gtcaactgga 180

ccacatttca atcctgctgg caaagagcat ggtgctcctg aggatgcgaa tcgtcatgct 240

ggtgatctgg gaaatgtcaa tgttggtgat gatggcacag tcagtttcac aataattgac 300

aaacagattc cactttgtgg tccaaattcc attatcggaa gggctgttgt tgtccatgga 360

gatccagatg atcttggcaa ggggggacat gaacttagca agagcactgg aaatgctggt 420

ggccgtatag cttgtggtat cattggtctc caaggatga 459

<210> SEQ ID NO 41
<211> LENGTH: 400
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 41

tctttaccca agagggagat ggcccaacta ctgtgaccgg aaacatttct ggcctcaagc 60

ctgggcttca tggtttccat gttcatgctc ttggggacac aaccaatggt tgcatgtcaa 120

ctggaccaca tttcaatcct gctggcaaag agcatgggtc tcctgaagat gagactcgtc 180

atgctggtga tcttggaaat atcactgttg gggatgacgg aactgcttgc ttcacaattg 240

ttgacaaaca gattcctctc actggaccac actctatcat tggtagggct gttgttgtcc 300

atgcagatcc tgatgacctt ggcaagggtg gacatgagct tagcaaatcc actggaaatg 360

ctggtggcag gatagcttgt ggtattattg gccttcaagg 400

<210> SEQ ID NO 42
<211> LENGTH: 400
<212> TYPE: DNA
<213> ORGANISM: Populus tremuloides

<400> SEQUENCE: 42

tctttaccca agaaggagat ggcccaacta ctgtaattgg aaacctttct ggtcttaagc 60

caggccttca tggcttccac gtccatgccc ttggagacac cacaaatggc tgcatgtcaa 120

ctgggccgca ttttaatcct gtaggcaagg agcatggtgc ccctgaggat gagaatcgtc 180

atgctggtga tctgggaaat gtcactgttg gtgatgatgg cactgctgct ttcacaatca 240

ttgacaaaca gattcctctt actggaccac attccattat tggttgggct gttgttgttc 300

atggagatcc tgatgatctt ggcaagggag gacatgaact cagcaaaacc actggtaatg 360

ctggcggcag agtagcatgc ggtattattg gtctgcaagg 400

<210> SEQ ID NO 43
<211> LENGTH: 416
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

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<400> SEQUENCE: 43

agtgagggtg ttgctggaac tatcctcttt acccaagagg gagatggccc aactactgtg 60

accggaaaca tttctggcct caagcctggg cttcatggtt tccatgttca tgctcttggg 120

gacacaacca atggttgcat gtcaactgga ccacatttca atcctgctgg caaagagcat 180

gggtctcctg aagatgagac tcgtcatgct ggtgatcttg gaaatatcac tgttggggat 240

gacggaactg cttgcttcac aattgttgac aaacagattc ctctcactgg accacactct 300

atcattggta gggctgttgt tgtccatgca gatcctgatg accttggcaa gggtggacat 360

gagcttagca aatccactgg aaatgctggt ggcaggatag cttgtggtat tattgg 416

<210> SEQ ID NO 44
<211> LENGTH: 416
<212> TYPE: DNA
<213> ORGANISM: Manihot esculenta

<400> SEQUENCE: 44

agtgagggtg ttgctgggac aatcttcttc acccaagaag gagatggtcc aaccaccgtc 60

actggaagtg tttctggcct taagccaggg cttcatggat tccatgttca tgcccttgga 120

gacacaacaa atggttgcat gtcaactggg ccacatttca accctggtgg caaagagcat 180

ggtgcccctg aggacgacat tcgtcatgct ggtgatctgg gaaatgtcac tgctggtgat 240

gatggcactg ctagtttcac aatcgttgac aaggatattc ctctttctgg tccgcattcc 300

attgtaggaa gggcagtcgt tgttcatgca gatcctgatg atcttggaaa gggggacat 360

gaacttagca aaaccactgg aaatgctggt ggcagggtag catgtggtgt tattgg 416

<210> SEQ ID NO 45
<211> LENGTH: 331
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 45

actatcctct ttacccaaga gggagatggc ccaactactg tgaccggaaa catttctggc 60

ctcaagcctg ggcttcatgg tttccatgtt catgctcttg gggacacaac caatggttgc 120

atgtcaactg gaccacattt caatcctgct ggcaaagagc atgggtctcc tgaagatgag 180

actcgtcatg ctggtgatct tggaaatatc actgttgggg atgacggaac tgcttgcttc 240

acaattgttg acaaacagat tcctctcact ggaccacact ctatcattgg tagggctgtt 300

gttgtccatg cagatcctga tgaccttggc a 331

<210> SEQ ID NO 46
<211> LENGTH: 331
<212> TYPE: DNA
<213> ORGANISM: Betula pendula

<400> SEQUENCE: 46

actatccact ttacccaaga agctgatggc ccaactacag taactggaaa tatttctggc 60

cttaagcctg ggctccatgg gttccatgtc catgcacttg gggacacaac aaatggttgc 120

atgtcaactg ggccacattt caatcctgct ggcaaagagc atggtgctcc tgaggatgag 180

aatcgtcatg ccggtgatct gggaaatgtc accgttggtg atgatggtac tgccagtttc 240

acaatagttg acaagcagat tccactttct ggaccacatt ctattattgg aagggctgtt 300

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gttgtccacg gggatccaga tgatcttggc a 331

<210> SEQ ID NO 47
<211> LENGTH: 390
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 47

ctttacccaa gagggagatg gcccaactac tgtgaccgga aacatttctg gcctcaagcc 60

tgggcttcat ggtttccatg ttcatgctct tggggacaca accaatggtt gcatgtcaac 120

tggaccacat ttcaatcctg ctggcaaaga gcatgggtct cctgaagatg agactcgtca 180

tgctggtgat cttggaaata tcactgttgg ggatgacgga actgcttgct tcacaattgt 240

tgacaaacag attcctctca ctggaccaca ctctatcatt ggtagggctg ttgttgtcca 300

tgcagatcct gatgaccttg gcaagggtgg acatgagctt agcaaatcca ctggaaatgc 360

tggtggcagg atagcttgtg gtattattgg 390

<210> SEQ ID NO 48
<211> LENGTH: 390
<212> TYPE: DNA
<213> ORGANISM: Populus tremula x Populus tremuloides

<400> SEQUENCE: 48

ctttacccaa gaaggagatg gtccaactac tgtaactgga agcctctgtg gtcttaagcc 60

aggccttcat ggcttccatg ttcatgccct tggagacacc acaaatggct gcatgtcaac 120

tggcccgcat tttaatcctg taggcaaaga gcatggtgcc cctgaggatg agaatcgtca 180

tgctggtgat ttgggaaatg tcactgttgg tgatgatggc accgctactg tctcaatcat 240

tgacaaccag attcctctca ctggaccaaa ttccatcgtt ggaagggctg ttgttgttca 300

tgcagatcct gatgatcttg gcaagggagg acatgaactt agcaaaagca ctggtaatgc 360

tggtggcaga gtagcatgtg gtgttattgg 390

<210> SEQ ID NO 49
<211> LENGTH: 446
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 49

aagggcgttg ctgtacttag ctccagtgag ggtgttgctg gaactatcct ctttacccaa 60

gagggagatg gcccaactac tgtgaccgga aacatttctg gcctcaagcc tgggcttcat 120

ggtttccatg ttcatgctct tggggacaca accaatggtt gcatgtcaac tggaccacat 180

ttcaatcctg ctggcaaaga gcatgggtct cctgaagatg agactcgtca tgctggtgat 240

cttggaaata tcactgttgg ggatgacgga actgcttgct tcacaattgt tgacaaacag 300

attcctctca ctggaccaca ctctatcatt ggtagggctg ttgttgtcca tgcagatcct 360

gatgaccttg gcaagggtgg acatgagctt agcaaatcca ctggaaatgc tggtggcagg 420

atagcttgtg gtattattgg ccttca 446

<210> SEQ ID NO 50
<211> LENGTH: 446
<212> TYPE: DNA
<213> ORGANISM: Plantago major

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<400> SEQUENCE: 50

aagggtgttg cagtgcttag cagcagtgag ggtgttagtg gcaccgtcct cttttcccaa 60

gaaggagaag gacccaccac tgtaactgga aacctttctg gccttaagcc tggacttcac 120

ggcttccatg ttcatgctct tggtgacact accaacggtt gcatgtcaac aggaccacat 180

ttcaatccgg ctgcaaaaga gcatggtgct cctgatgatg aggttcgcca tgctggtgac 240

cttggtaatg tcacagtggg agatgatgga actgcaagtt tcaccattgt tgacaagctg 300

attccgctga ctggaccaca ttccatcatt ggaagggctg ttgttgtcca tgctgacccc 360

gatgatttgg gaaggggtgg acatgaactc agcaaaacta ccggaaatgc tggtggaaga 420

gttgcttgtg gtatcattgg tcttca 446

<210> SEQ ID NO 51
<211> LENGTH: 401
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 51

ggaactatcc tctttaccca agagggagat ggcccaacta ctgtgaccgg aaacatttct 60

ggcctcaagc ctgggcttca tggtttccat gttcatgctc ttggggacac aaccaatggt 120

tgcatgtcaa ctggaccaca tttcaatcct gctggcaaag agcatgggtc tcctgaagat 180

gagactcgtc atgctggtga tcttggaaat atcactgttg gggatgacgg aactgcttgc 240

ttcacaattg ttgacaaaca gattcctctc actggaccac actctatcat tggtagggct 300

gttgttgtcc atgcagatcc tgatgacctt ggcaagggtg gacatgagct tagcaaatcc 360

actggaaatg ctggtggcag gatagcttgt ggtattattg g 401

<210> SEQ ID NO 52
<211> LENGTH: 401
<212> TYPE: DNA
<213> ORGANISM: Manihot esculenta

<400> SEQUENCE: 52

ggaacaatct tctttaccca agaaggagat ggtcctacca ctgtaactgg aaacatttcc 60

ggccttaagc cagggcttca tgggttccac gtccatgccc ttggagacac aacaaacggt 120

tgcatgtcaa ctgggccaca ctttaaccct tctggcaaag atcatggtgc ccctgaggat 180

gagattcgtc atgctggtga tctgggaaat gtcactgctg gtgatgatgg cactgctagt 240

ttcacaatta ttgacaagca tattcctctt tctggtcaaa attcaatcat aggaagggca 300

gttgttgttc atgcagatcc tgatgatctt ggcaggggag gacatgaact cagtaaaacc 360

accggaaatg ctggtggcag agtagcatgc ggtattattg g 401

<210> SEQ ID NO 53
<211> LENGTH: 398
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 53

tttacccaag agggagatgg cccaactact gtgaccggaa acatttctgg cctcaagcct 60

gggcttcatg gtttccatgt tcatgctctt ggggacacaa ccaatggttg catgtcaact 120

ggaccacatt tcaatcctgc tggcaaagag catgggtctc ctgaagatga gactcgtcat 180

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gctggtgatc ttggaaatat cactgttggg gatgacggaa ctgcttgctt cacaattgtt 240

gacaaacaga ttcctctcac tggaccacac tctatcattg gtagggctgt tgttgtccat 300

gcagatcctg atgaccttgg caagggtgga catgagctta gcaaatccac tggaaatgct 360

ggtggcagga tagcttgtgg tattattggc cttcaagg 398

<210> SEQ ID NO 54
<211> LENGTH: 398
<212> TYPE: DNA
<213> ORGANISM: Condonopsis lanceolata

<400> SEQUENCE: 54

tttacccaag agggagatgg cccaactaaa gttactggaa gcctttctgg ccttcaacct 60

ggacctcacg gtttccatgt tcatgccctt ggtgacacaa ccaatggttg catgtcaact 120

ggtcctcatt ataatcctgc tggaaaagaa catggtgctc cagaggacga gattcgtcat 180

gctggtgacc tcgggaatgt tacagtaggc gaagacggta ctgcaaattt caccatcgtt 240

gacaaccaga ttccactatc tggacctcat tctatcattg gaagggctgt agttgtccat 300

gctgatcctg atgatcttgg aaagggtggc catgaactca gcaaaagcac tggaaatgct 360

ggtggcagga ttgcctgtgg tatcattgga ctgcaagg 398

<210> SEQ ID NO 55
<211> LENGTH: 394
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 55

cccaagaggg agatggccca actactgtga ccggaaacat ttctggcctc aagcctgggc 60

ttcatggttt ccatgttcat gctcttgggg acacaaccaa tggttgcatg tcaactggac 120

cacatttcaa tcctgctggc aaagagcatg ggtctcctga agatgagact cgtcatgctg 180

gtgatcttgg aaatatcact gttggggatg acggaactgc ttgcttcaca attgttgaca 240

aacagattcc tctcactgga ccacactcta tcattggtag ggctgttgtt gtccatgcag 300

atcctgatga ccttggcaag ggtggacatg agcttagcaa atccactgga aatgctggtg 360

gcaggatagc ttgtggtatt attggccttc aagg 394

<210> SEQ ID NO 56
<211> LENGTH: 394
<212> TYPE: DNA
<213> ORGANISM: Gossypium hirsutum
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (158)
<223> OTHER INFORMATION: a, c, g, t, unknown or other
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<222> LOCATION: (221)
<223> OTHER INFORMATION: a, c, g, t, unknown or other
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<222> LOCATION: (251)
<223> OTHER INFORMATION: a, c, g, t, unknown or other

<400> SEQUENCE: 56

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cccaagaagg agatggtcca actaccgtga ctgggaacct ttctggtctt aagccgggac 60

tccatggctt ccatgttcat gcccttgggg acacaactaa cgggtgcatg tcaactggac 120

cccattttaa tcctgctggc aaagagcatg gtgctccnga agatgagaac cgccatgctg 180

gtgatctagg naatgtcact gttggtgatg atggctgtgc nagcttctcc atcaccgaca 240

aacagattcc nctcacaggc ccaaactcca ttatcggaag agctgtagtt gtccatgcag 300

atcccgatga ccttggcaag ggcggccatg agctcagcaa aagcacagga aatgctggcg 360

gcagagtagc ttgcggtatt attggtctgc aagg 394

<210> SEQ ID NO 57
<211> LENGTH: 331
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 57

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tgggcttcat ggtttccatg ttcatgctct tggggacaca accaatggtt gcatgtcaac 120

tggaccacat ttcaatcctg ctggcaaaga gcatgggtct cctgaagatg agactcgtca 180

tgctggtgat cttggaaata tcactgttgg ggatgacgga actgcttgct tcacaattgt 240

tgacaaacag attcctctca ctggaccaca ctctatcatt ggtagggctg ttgttgtcca 300

tgcagatcct gatgaccttg gcaagggtgg a 331

<210> SEQ ID NO 58
<211> LENGTH: 331
<212> TYPE: DNA
<213> ORGANISM: Fagus sylvatica

<400> SEQUENCE: 58

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tggaccacat ttcaatcctg ctggcaaagg gcatggtgct cctgaggatg cgaatcgtca 180

tgctggtgat ctgggaaatg tcaatgttgg tgatgatggc acagtcagtt tcacaataat 240

tgacaaacag attccacttt gtggtccaaa ttccattatc ggaagggctg ttgttgtcca 300

tggagatcca gatgatcttg gcaagggtgg a 331

<210> SEQ ID NO 59
<211> LENGTH: 399
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 59

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tggaccacat ttcaatcctg ctggcaaaga gcatgggtct cctgaagatg agactcgtca 180

tgctggtgat cttggaaata tcactgttgg ggatgacgga actgcttgct tcacaattgt 240

tgacaaacag attcctctca ctggaccaca ctctatcatt ggtagggctg ttgttgtcca 300

tgcagatcct gatgaccttg gcaagggtgg acatgagctt agcaaatcca ctggaaatgc 360

tggtggcagg atagcttgtg gtattattgg ccttcaagg 399

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<210> SEQ ID NO 60
<211> LENGTH: 399
<212> TYPE: DNA
<213> ORGANISM: Citrus limon

<400> SEQUENCE: 60

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tggtcctcat ggattccatg ttcatgctct tggagacaca acaaatggtt gcatgtctac 120

tggaccccac tttaaccctg ctggaaaaga acatggagct ccagaggatg ataatcgtca 180

tgctggtgat ttaggaaatg tcaatgttag tgatgatggt actgctactt ttacagttgt 240

tgacaatcag attcctcttt ctggaccaaa ttccattatt ggaagggctg ttgtagtcca 300

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tggtggcaga gtagcttgcg gcataattgg cctccaagg 399

<210> SEQ ID NO 61
<211> LENGTH: 375
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 61

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acatttcaat cctgctggca aagagcatgg gtctcctgaa gatgagactc gtcatgctgg 180

tgatcttgga aatatcactg ttggggatga cggaactgct tgcttcacaa ttgttgacaa 240

acagattcct ctcactggac cacactctat cattggtagg gctgttgttg tccatgcaga 300

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caggatagct tgtgg 375

<210> SEQ ID NO 62
<211> LENGTH: 375
<212> TYPE: DNA
<213> ORGANISM: Bruguiera gymnorhiza

<400> SEQUENCE: 62

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gcacttcaat ccaggtagca aagagcatgg tgcccctgaa gacgagaacc gtcatgccgg 180

tgacctagga aatgtaaatg ttgcggatga tggcactgca acattcacaa tcactgacaa 240

tcagattcct cttactggac ccaattccat tgttggaagg gctgttgttg ttcatgctga 300

tcctgatgat ctgggcaagg gagggcatga acttagcaaa agcactggaa atgctggtgg 360

cagggtagca tgtgg 375

<210> SEQ ID NO 63
<211> LENGTH: 390
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 63

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tggaccacat ttcaatcctg ctggcaaaga gcatgggtct cctgaagatg agactcgtca 180

tgctggtgat cttggaaata tcactgttgg ggatgacgga actgcttgct tcacaattgt 240

tgacaaacag attcctctca ctggaccaca ctctatcatt ggtagggctg ttgttgtcca 300

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tggtggcagg atagcttgtg gtattattgg 390

<210> SEQ ID NO 64
<211> LENGTH: 390
<212> TYPE: DNA
<213> ORGANISM: Populus alba

<400> SEQUENCE: 64

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tggcccgcat tttaatcctg taggcaaaga gcatggtgcc cctgaggatg agaatcgtca 180

tgctggtgat ttgggaaatg tcactgttgg tgatgatggc accgctactg tctcaatcat 240

tgacaaccag attcctctta ctggaccaaa ttccattgtt ggaagggcag ttgttgttca 300

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tggtggcaga gtagcatgtg gtgttattgg 390

<210> SEQ ID NO 65
<211> LENGTH: 390
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 65

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tggaccacat ttcaatcctg ctggcaaaga gcatgggtct cctgaagatg agactcgtca 180

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tgacaaacag attcctctca ctggaccaca ctctatcatt ggtagggctg ttgttgtcca 300

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tggtggcagg atagcttgtg gtattattgg 390

<210> SEQ ID NO 66
<211> LENGTH: 390
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 66

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tggcccgcat tttaatcctg taggcaaaga gcatggtgcc cctgaggatg agaatcgtca 180

tgctggtgat ttgggaaatg tcactgttgg tgatgatggc accgctactg tctcaatcat 240

tgacaaccag attcctctta ctggaccaaa ttccattgtt ggaagggcag ttgttgttca 300

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tggtggcaga gtagcatgtg gtgttattgg 390

<210> SEQ ID NO 67
<211> LENGTH: 423
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 67

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ggacacaacc aatggttgca tgtcaactgg accacatttc aatcctgctg gcaaagagca 180

tgggtctcct gaagatgaga ctcgtcatgc tggtgatctt ggaaatatca ctgttgggga 240

tgacggaact gcttgcttca caattgttga caaacagatt cctctcactg gaccacactc 300

tatcattggt agggctgttg ttgtccatgc agatcctgat gaccttggca agggtggaca 360

tgagcttagc aaatccactg gaaatgctgg tggcaggata gcttgtggta ttattggcct 420

tca 423

<210> SEQ ID NO 68
<211> LENGTH: 423
<212> TYPE: DNA
<213> ORGANISM: Populus alba

<400> SEQUENCE: 68

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tactggaaac gtttctggcc ttaagcctgg accccatggc tttcatgtgc atgcccttgg 120

tgacaccacc aatggttgtt tgtcaactgg acctcacttc aatcctgctg gcaaagagca 180

tggagctcct gatgatgagg ttcgccatgc tggtgacctt gggaatgtca cagttggaga 240

agatggcact gctgctttca ctattgttga caagcagata ccacttacag gaccacattc 300

cataattgga agagctgtag ttgttcatgc tgatcctgat gatcttggaa agggtggaca 360

tgaactgagc aaaaccactg gaaatactgg tggaagagtt gcttgtggta tcaatggcct 420

tca 423

<210> SEQ ID NO 69
<211> LENGTH: 392
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 69

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cttcatggtt tccatgttca tgctcttggg gacacaacca atggttgcat gtcaactgga 120

ccacatttca atcctgctgg caaagagcat gggtctcctg aagatgagac tcgtcatgct 180

ggtgatcttg gaaatatcac tgttggggat gacggaactg cttgcttcac aattgttgac 240

aaacagattc ctctcactgg accacactct atcattggta gggctgttgt tgtccatgca 300

gatcctgatg accttggcaa gggtggacat gagcttagca aatccactgg aaatgctggt 360

ggcaggatag cttgtggtat tattggcctt ca 392

<210> SEQ ID NO 70
<211> LENGTH: 392

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<212> TYPE: DNA
<213> ORGANISM: Olea europaea

<400> SEQUENCE: 70

acccaagaag gagatggtcc aactactgtt actggaaacc tttctggcct taagcctgga 60

cttcatggct ttcatgtcca cgcccttggt gacaccacca atggctgtat gtcaactgga 120

cctcatttca atcctgttgg gaaagagcat ggtgcacctg gagatgagaa ccgtcatgct 180

ggtgatcttg gtaatatcac agttggcgaa gatggcaccg ctgctatcaa cattgttgac 240

aagcagatac ctcttacagg accacattcc ataattggaa gagcagtagt tgtccattca 300

gatcctgatg atcttggaag gggtggtcat gaactgagca agagcactgg aaatgctggt 360

ggaagagttg cttgtggtat cattggcctt ca 392

<210> SEQ ID NO 71
<211> LENGTH: 395
<212> TYPE: DNA
<213> ORGANISM: Potentilla atrosanguinea

<400> SEQUENCE: 71

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aagcctgggc ttcatggttt ccatgttcat gctcttgggg acacaaccaa tggttgcatg 120

tcaactggac cacatttcaa tcctgctggc aaagagcatg ggtctcctga agatgagact 180

cgtcatgctg gtgatcttgg aaatatcact gttggggatg acggaactgc ttgcttcaca 240

attgttgaca aacagattcc tctcactgga ccacactcta tcattggtag ggctgttgtt 300

gtccatgcag atcctgatga ccttggcaag ggtggacatg agcttagcaa atccactgga 360

aatgctggtg gcaggatagc ttgtggtatt attgg 395

<210> SEQ ID NO 72
<211> LENGTH: 395
<212> TYPE: DNA
<213> ORGANISM: Solanum tuberosum

<400> SEQUENCE: 72

atcctcttca ctcaagatgg agatgctcca accacagtta atggaaatat ttctggccta 60

aaacctggac ttcatggctt ccatgtccat gcccttggtg ataccacaaa tggctgcatg 120

tcaacaggac cacattacaa tcctgctggt aaggagcatg gtgctcctga agatgaggtg 180

cgtcatgctg gtgatcttgg taacatcaca gttggagaag atggtactgc atcttttact 240

attaccgaca agcagattcc tctcactggt tcacaatcca tcattggaag agctgttgtt 300

gttcatgctg atcctgatga tcttggaaag ggaggacatg agctcagtaa aagcactgga 360

aatgctggcg gaaggattgc ttgtggtatt attgg 395

<210> SEQ ID NO 73
<211> LENGTH: 57
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic oligonucleotide
<220> FEATURE:
<221> NAME/KEY: modified_base
<222> LOCATION: (56)..(57)
<223> OTHER INFORMATION: a, c, g, t, unknown or other

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<400> SEQUENCE: 73

aagcagtggt atcaacgcag agtacttttt ttttttttt ttttttttt tttttnn 57

We claim:

1. Superoxide dismutase (SOD) cDNA of SEQ ID No. 2 obtained from *Potentilla atrosanguinea,* wherein the said cDNA comprising of 856 nucleotide bases.

2. Superoxide dismutase (SOD) cDNA as claimed in claim 1, wherein the said cDNA has entire coding sequence along with pre- and post-coding sequences.

3. Superoxide dismutase (SOD) gene coding cDNA of SEQ ID No. 3, wherein the said coding cDNA comprising of 459 nucleotide bases.

4. Superoxide dismutase (SOD) polypeptide of SEQ ID No. 1, wherein the said polypeptide comprising of 152 amino acids.

5. Superoxide dismutase (SOD) polypeptide as claimed in claim 4, wherein the said polypeptide is autoclavable.

6. Superoxide dismutase (SOD) polypeptide as claimed in claim 4, wherein the said polypeptide is functional at temperature range of <−10° C. to +80° C.

7. A set of primers useful for the amplification of Superoxide dismutase (SOD) gene coding cDNA of SEQ ID No. 3, wherein

Forward primer 5'-ATGGCAAAGGGCGTTGCTG-TACTT-3' (SEQ ID NO: 5) and;

Rreverse primer 5'-TCATCCTTGMGGCCMT-MTACCA-3' (SEQ ID NO: 6)

8. A method of identifying and cloning of superoxide dismutase (SOD) gene of SEQ ID NO 3 which codes for a polypeptide of SEQ ID No. 1 having Superoxide dismutase enzyme activity, wherein the said method comprising the steps of:

a) isolating the mRNA from leaves of *potentialla;*

b) synthesizing the cDNA from mRNA as obtained from step (a);

c) constructing a cDNA library of the DNA of *potentilla* followed by the cloning of the cDNA obtained from step (b) in a suitable vector preferably in bacteriophage;

d) screening the said library obtained from step (c) followed by the primary, secondary and tertiary screening for identification of positive cDNA clones;

e) isolating the DNA from positive cDNA clones obtained from step (d);

f) amplifying the said DNA using the primers comprising:

Forward Primer:
5'-GTTGTAAAACGACGTGCCAGT-3' (SEQ ID NO: 13)

Reverse Primer:
5'-CACAGGAAACAGCTATGACC-3'; (SEQ ID NO: 14)

h) amplifying the ends of cDNA obtained from step (e) through rapid amplification of cDNA ends technique (RACE) using different set of primers to get the full length desired Superoxide dismutase (SOD) DNA of SEQ ID NO. 2 wherein the said primers comprising:

Forward Primer (GSP1):
(SEQ ID NO: 7)
5'-CCAGTGGATTTGCTAAGCTCATGTCCA-3'

Reverse Primer (NES1):
(SEQ ID NO: 8)
5'-GTCATCAGGGTCTGCATGGACAACAAC-3'

Forward Primer (GSP2):
(SEQ ID NO: 9)
5'-ATGGTTGCATGTCAACTGGACCACATT-3'

Reverse Primer (NES2):
(SEQ ID NO: 10)
5'-TTGCATGTCAACTGGACCACATTTCAA-3'

SMART II A Oligonucleotide:
(SEQ ID NO: 11)
5'AAGCAGTGGTATCAACGCAGAGTAC GCGGG-3'

3'- RACE CDS Primer A (3'- CDS):
(SEQ ID NO: 73)
5'AAGCAGTGGTATCAACGCAGA
GTAC $(T)_{30}$ N_{-1}N-3'

5'- RACE CDS Primer (5'- CDS)
(SEQ ID NO: 15)
5'- $(T)_{25}$ N_{-1}N- 3'

Universel Primer Mix A (UPM): Long:
(SEQ ID NO: 16)
5'TAATACGACTCACTATAGGGC
AAGCAGTG GTATCAACGCAGAGT-3'

Universel Primer Mix A (UPM): Short:
(SEQ ID NO: 17)
5'-CTAATACGACTCACTATAGG
GC-3'

Nested Universal Primer A (NUP):
(SEQ ID NO: 23)
5'-AAGCAGTGGTATCAACGCAGAGT-3'

i) amplifying the coding sequence of Superoxide dismutase (SOD) of SEQ ID No. 3 using a set of primers designed from start and stop codon of full length desired Superoxide dismutase (SOD) DNA of SEQ ID NO. 2 wherein the said primers have the following sequences:

Forward Primer:
5'-ATGGCAAAGGGCGTTGCTGTACTT-3' (SEQ ID NO: 5)

Reverse Primer:
5'-TCATCCTTGAAGGCCAATAATACCA-3' (SEQ ID NO: 6)

j) cloning the amplified product obtained from step (g) into pQE 30 expression vector followed by the transformation it into competent *E.coli* cells to get an expression construct;

k) isolating the plasmid DNA by conventional method followed by sequencing to confirm the said SOD gene.

9. A method as claimed in claim 9, wherein the polyclonal antibodies were raised against the purified SOD and used for cDNA library screening synthesized from young leaf mRNA.

10. A method as claimed in claim 9, wherein the 10^5 plaque forming units (pfu) are taken for primary screening.

11. A method as claimed in claim 11, wherein three strong positive clones are obtained from the primary cloning.

12. A method as claimed in claim 12, wherein the said positive clones are taken for secondary screening which gives about 70% positive clones.

13. A method as claimed in claim 13, wherein the said positive clones are randomly taken for tertiary screening which gives 100% positive signal after tertiary screening.

14. A method as claimed in claim 9, wherein the said RACE Primers are designed such that the amplified 5' and 3'end overlap each other over a small stretch of nucleotides.

15. A method as claimed in claim 9, wherein the said full length SOD gene of SEQ ID No. 2 contains 856 nucleotide bases.

16. A method as claimed in claim 16, wherein the said full length SOD gene of SEQ ID No. 2 has entire coding sequence along with pre- and post-coding sequences

17. A method as claimed in claim 9, wherein the said coding cDNA of SEQ ID No. 3 comprises 459 nucleotide bases.

18. A method as claimed in claim 18, wherein the said coding sequence of SOD gene of SEQ ID No. 3 corresponding to polynucleotides encoding Superoxide dismutase (SOD) enzyme.

19. An expression construct comprises a nucleotide sequence of superoxide dismutase (SOD) gene of SEQ ID NO 3 which codes for a polypeptide of SEQ ID No. 1 having Superoxide dismutase enzyme activity, a selectable marker and a terminator sequence.

* * * * *