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ABSTRACT
Superoxide dismutase has important roles to detoxify reactive oxygen species toxicity in all aerobic organisms. Also it's expression increase under abnormal and stressful conditions. In this research genetics, amino acids and promoter of Cu/Zn Superoxide dismutase for human and Arabidopsis plant analyzed. Raw data collected from NCBI and Uniprot websites. Map viewer results indicated that this gene localized on chromosome 21, it has 5 exons and 4 introns, but in Arabidopsis Cu/Zn SOD exist on chromosome 21 and has 8 introns. Also indicated binding sites and variations and network display of two Cu/Zn SODs with other molecules. Promoter analyses indicated that these genes have some transcription factors such as early responsive to dehydration and ABA-responsive that important to oxidative stress tolerance. MEME analyzed showed that there are important motifs such as GLHGFHVH, GPHFNP and DDLGKGG which conserved under evolution. It seems that this study results can be useful for protein engineering and production of transgenic organisms.

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Introduction

In plants and animals, reactive oxygen species (ROS) can act as a signaling molecules (low content) or toxic compounds (high content) which lead to oxidative damage (Martin et al. 2013). Stresses such as salinity, drought, cold, heat, etc. have a negative impact on gaseous exchange. This phenomena decrease plants CO₂ content for photosynthesis, subsequently decrease electron cycle transportation and produce ROS (Mateo et al. 2004; Modarresi et al. 2013b), such as superoxide anion and hydrogen peroxide (Ben Amor et al. 2005; Modarresi et al. 2013a). On the other hand, in mammalian cells, ROS is produced by some cellular metabolisms such as mitochondrial respiration (Waris and Ahsan 2006). ROS can damage DNA, lipids, proteins and cell division lead to mutations or tumors such as breast cancer (Jaiyesimi et al. 1992), colorectal cancer (Sreevalsan and Safe 2013), lung cancer (Manda et al. 2009) etc.

Aerobian organisms employ different mechanisms such as antioxidant enzymes to scavenge ROS. Superoxide dismutases (SODs, EC 1.15.1.1) are the first line of defense against free oxygen radicals within the cells. These
enzymes scavenge superoxide anion into hydrogen peroxide and oxygen molecule (Deeba et al. 2012; Modarresi et al. 2012). Superoxide dismutase is divided into three categories (copper/zinc (Cu/Zn SOD), manganese (Mn SOD) and iron (Fe SOD)), based on the type of metal used in the cofactor of the enzyme. Various types of Cu/Zn SODs have been known; cytoplasmic and chloroplastic forms in eukaryotes and a periplasmic form in prokaryotes. Extracellular SOD expression is higher than Mn and intracellular Cu/Zn SODs. It exist in mammalian fluids, lymph and plasma. Its expression depend on position of secreting cells in tis tissues (Zelko et al. 2002). Mn SOD exists in mitochondria of aerobic organisms as a cofactor and it has a major role for cellular tumorgenesis control (Chen et al. 2013).

Mammals only have Cu/Zn SODs (intracellular and extracellular) and Mn SODs, but some plants such as Arabidopsis have all three groups of superoxide dismutase enzymes.

Superoxide dismutase genes have an important role in combating disease and tolerant or sensitivity to environmental stress such as salinity and drought, so in this research variation among Cu/Zn superoxide dismutase genes from different species has been investigated by bioinformatics analysis.

**Materials and Methods**

Database searching were carried out using the Uniprot website (available at www.uniprot.org), Genebank programs (available at http://www.ncbi.nlm.nih.gov/) and PHYTOZOME website available at (www.phytozome.net). Protein sequence motifs identified by MEME program (available at http://meme.nbcr.net), Polypeptide domains determined by SMART program (www.smart.embl-heidelberg.de). DnaSP software also used for codon usage and haplotype determination.

**Results and Discussion**

Superoxide dismutase enzymes destroy radicals which are toxic to biological systems and are produced under stressful or normally conditions within the cells. All Cu/Zn superoxide dismutase gene sequences obtained from NCBI databank and genes position in chromosomes determined by Map viewer program. Arabidopsis thaliana has seven SODs, including three Cu/Zn SODs (on chromosome 1, 2 and 5); three Fe SODs (on chromosomes 4 and 5) and one Mn SOD (on chromosome 3), but Homo sapiens SODs localized on chromosomes 21 (Cu/Zn), Mn SOD on chromosome 6 and another type Cu/Zn SOD (extracellular spaces) exists on chromosome 4.

Human Cu/Zn SOD and Mn SOD have 5 exons and 4 introns, meanwhile extracellular Cu/Zn SOD has 3 exons and 2 introns. Arabidopsis Cu/Zn gene contains 8 different gt-ag introns, furthermore transcription produces 3 alternatively spliced mRNAs. It seems that alternative splicing accurse among different mRNA’s exons or one intron remain in final mRNAs.

**Table 1. Superoxide dismutase sequences utilized in this research**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Proposed location</th>
<th>Species</th>
<th>Length (aa)</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu/Zn SOD</td>
<td>Cytoplasm, Nucleus</td>
<td>Arabidopsis thaliana</td>
<td>152</td>
<td>X60935.1</td>
</tr>
<tr>
<td>Cu/Zn SOD</td>
<td>Cytoplasm, Nucleus</td>
<td>Homo sapiens</td>
<td>154</td>
<td>NM_000454.4</td>
</tr>
</tbody>
</table>
ProtParam analyses (available at: web.expasy.org/protParam) indicated that Arabidopsis Cu/Zn SOD protein has 152 aa, 150.97 KDa mass, Theoretical pI: 5.24, total number of negatively charged residues (Asp + Glu): 15, total number of positively charged residues (Arg + Lys): 7, Formula: C_{646}H_{1023}N_{191}O_{217}S_{5}, total number of atoms: 2082. Instability index (II) is computed to be 14.73 (this classifies the protein as stable), grand average of hydropathicity (GRAVY): -0.076 and aliphatic index: 76.32. In other hand, human Cu/Zn SOD protein has 154 aa, 159.97 KDa mass, Theoretical pI: 5.7, total number of negatively charged residues (Asp + Glu): 21, total number of positively charged residues (Arg + Lys): 15, Formula: C_{684}H_{1094}N_{204}O_{225}S_{5}, total number of atoms: 2212 and finally instability index (II) is computed to be 21.62 (this classifies the protein as stable), grand average of hydropathicity (GRAVY): -0.344 and aliphatic index: 78.44.

The metal binding sites are conserved in all the known Cu/Zn SODs sequences (Smith and Doolittle 1992). In the Cu/Zn Superoxide dismutase one copper and zinc ions bind per each subunit. Uniprot data (available at http://www.uniprot.org/uniprot/P00441) indicated that human Cu/Zn SOD (Table 1) has several metal binding sites; Copper (catalytic), Zinc (via pros nitrogen), Zinc (structural) and Copper (catalytic) attached to amino acids at the positions 47, 49 and 64; 64, 72 and 81; 84; 121; respectively. Arabidopsis thaliana Cu/Zn SOD usually expressed in leaves and pollen at protein level. EST Profile (available at http://www.ncbi.nlm.nih.gov/unigene) indicated that the Cu/Zn SOD often exists in buds, floral meristems, flowers and old rosette leaves; and to a lower extent, in stems, siliques and roots; and is not exist in seeds. Its expression induced by UV-B, copper ion, light intensity, sucrose stimulus, ozone fumigation, oxidative stress, iron ion, salt stress and defense response to bacterium (Attia et al. 2008; Sunkar et al. 2006). It has 152 amino acids (two amino acids less than human Cu/Zn SOD). Arabidopsis and human Cu/Zn SODs have some conserve sequences such as GLHGFVHV, GPHFNP and DDLGKGG that preserved in evolution process (Figure 1).

Figure 1. Multiple alignments of Cu/Zn Superoxide dismutase genes between Arabidopsis thaliana (Accession No. X60935.1) and Homo sapiens (Accession No. NM_000454.4).
MEME software results indicated that Arabidopsis and Human Cu/Zn SODs have five major motifs including LHGFHVH[AE][FL] GD[NT]I[AN][GC][MT][S][AT]GPHFNP[DL][GS][KR][KT]HG[AG]P[EK]D[AE][EN]RH, I[ET]D[CS][QV][L][PS][L][ST][G][DP][HN][CS][I[V][GR][AT][LV][V][H][AE][DK][AP]DDLGBK [HN][E][EL][S][LT][AK][TGNAG][GS][R][LV][AC, I[FN][F][ET][Q][EK], MA[KT][GK][AV][AV] and DLGN[IV]T (Figure 2).

ScanProsite program (available at www.prosite.expasy.org/scanprosite/) found two different hits in Arabidopsis thaliana amino acids sequence; GFHVHAIGDIT (at the positions 43-53) and GNAGGvACgI (at the positions 138-147). They are Copper/Zinc superoxide dismutase signatures with consensus patterns [GA]-[IMFAT]-H-[LIVF]-H-[S] a-x-[GP]-[SDG]-x-[STAGDE] and G-[GNHD]-[SGA]-[GR]-x-R-x-[SGAWRV]-C-x(2)-[IV], respectively. Also these hints found in position 45-55 and 139-150 in human Cu/Zn Superoxide dismutase amino acids sequence. Plant Care (available at http://bioinformatics.psb.ugent.be/webtools/plantcare/html/search_CARE.html) and PLACE programs (available at http://www.dna.affrc.go.jp/PLACE/) used for Arabidopsis and human promoter region analyses, respectively. Results indicate that there are some regulatory elements such as ABRE-like, A-box, ACGT sequence, amylase box (in Arabidopsis) and AP-2 and SP1 (in human) (Table 2). DnaSP analyses indicated that Arabidopsis and human Cu/Zn SODs nucleotides have 321 polymorphic sites. Also Nucleotide diversity (Pi) is 0.69935, Variance of theta (no recombination) is 0.2453045, stochastic variance of k (no recombination) is 11520.333, Number of pairwise comparisons analyzed is 51360, Singleton variable sites are 321 and Invariable (monomorphic) sites are 138.

| Table 2. Arabidopsis thaliana and Homo sapiens Cu/Zn SODs genes promoter region motifs |
|-----------------------------------------------|----------------|-----|-----------------|
| Species | Motifs | Position | Sequence | Function |
| Arabidopsis thaliana | -10PEHVPB | 157 | TATTTCT | -10 promoter element, activated blue, white or UV-A light |
| Arabidopsis thaliana | ABRELATERD1 | 1387 | ACGTG | early responsive to dehydration |
| Arabidopsis thaliana | ACGTABOX | 62 | TACGTA | responsible for sugar repression |
| Arabidopsis thaliana | ACGTABREMOTIFAOSEM | 1387 | ACGTGC | ABA-responsive |
| Arabidopsis thaliana | ACGTATERD1 | 63 | ACGT | early responsive to dehydration |
| Homo sapiens | TATA box | 1157 | TATAAAAA | Promoter Position |
| Homo sapiens | Sp1-hsp70 | 2368 | GGCCTGG | proximal site is capable of response to Sp1-dependent stimulation |
| Homo sapiens | AP-2 CS6 | 2433 | CCCMNSSS | vertebrate-encoded transcription factor |
| Homo sapiens | GCF CS | 2355 | SCGSSSSC | Vertebrate-encoded trans factors |

STRING 9.05 program (available at string905.embl.de) used for prediction network display of Arabidopsis thaliana and Homo sapiens Cu/Zn SODs (Figure 3). Data indicated that Arabidopsis thaliana Cu/Zn SOD have interaction with CSD2 (encodes a chloroplastic
Copper/Zinc superoxide dismutase 2); superoxide dismutase; CSD3 (peroxisomal Copper/Zinc superoxide dismutase 3), WD-40 repeat family protein (transducin family protein) and PHD finger family protein.

**Figure 2.** Motifs for Cu/Zn Superoxide dismutases proteins. The MEME motifs are shown as different-colored boxes. Biochemical properties of the various amino acids indicated: Blue; most hydrophobic, Magenta; acidic, Red; positively charged and Green; Polar, non-charged and non-aliphatic residues.

Data indicated that *Arabidopsis thaliana* Cu/Zn SOD have interaction with CSD2 (encodes a chloroplastic Copper/Zinc superoxide dismutase 2); superoxide dismutase; CSD3 (peroxisomal Copper/Zinc superoxide dismutase 3), WD-40 repeat family protein (transducin family protein) and PHD finger family protein. But *Homo sapiens* Cu/Zn SOD has interactions with mitochondrial superoxide dismutase 2, ubiquitin C, catalase, ring finger protein 19A, ring finger protein 19A, estrogen receptor 1 and heat shock 70kDa protein 4.

**Conclusion**

Aerobic organisms utilized many different methods to destroy reactive oxygen species produced under normal and stress conditions. Superoxide dismutase is one of the important genes that removed superoxide anions from plants and animal cells. We analyzed Arabidopsis and human Superoxide dismutase nucleotides, amino acids and promoter regions for finding their evolutionary relationship.
Our experiment indicated that some parts of this gene conserved and maintained in evolution. Also promoter analyses indicated some transcription factors which related to tolerance to stressfully conditions such as dehydration or salinity. Results of this research can be useful for protein engineering and production transgenic plants or other organisms for tolerance to an oxidative stresses.

References