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Salinity response pattern and isolation of catalase gene from halophyte plant *Aeluropus littoralis*

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Abstract

The role of the antioxidant defense system in salt tolerance of *Aeluropus littoralis* has not been yet reported; therefore in the present study, the changes of catalase (CAT) activity in this halophyte plant was investigated and CAT gene was isolated. The leaves of treated and control plants were harvested at various times, starting 1 day prior to initiating treatment, then periodically at 72-h intervals for 21 days. The data collected showed that CAT activity increased significantly with time in plants treated with 200, 400, and 600 mM NaCl when compared with the control plants. Maximum enzyme activity was observed between the 6th and 12th day at all NaCl concentrations. CAT gene was isolated and cloned *via* pTZ57R/T cloning vector in *Escherichia coli*. CAT gene encoded 494 amino acids and had also high homology of 90, 87, 86, and 86% with CAT genes from *Zea mays*, *Oryza sativa, Triticum aestivum*, and *Hordeum vulgare*, respectively.

Additional key words: bioinformatic analysis*;* catalase activity; gene isolation.

Introduction

Salinity is one of the most severe problems in agricultural production (Castelli *et al.* 2010, Modarresi *et al.* 2013). About 800 million ha of land throughout the world is already affected by salinity. High salinity has ionic, osmotic, and secondary negative impacts, such as hormonal and nutritional imbalances (Rubio *et al.* 2009, Seckin *et al.* 2010). In addition to ionic and osmotic components, salt stress also leads to oxidative stress through an increase in reactive oxygen species (ROS), such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) (Mittler 2002, de Azevedo Neto *et al.* 2006). Plants possess several antioxidant enzyme systems to protect their cells from potential cytotoxic effects of ROS (Garratt *et al.* 2002). For instance, plants use a diverse array of enzymes, such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), peroxidase (POD; EC 1.11.1.7), and ascorbate peroxidase (APX; EC 1.11.1.11) to scavenge different types of ROS (Apse and Blumwald 2002, Vaidyanathan *et al.* 2003). CAT is the key enzyme for scavenging H_2O_2 by its dismutation to O_2 and H_2O. The induction of CAT in response to different, stressful environments reflects its important role in plant defense mechanisms (Purev *et al.* 2010).

Perennial halophyte, *A. littoralis*, is a salt-emitting, rhizomatous grass, ranging in its distribution from Morocco in Northern Africa and Sicily in the Mediterranean through the Middle East to Central Asia, Iran, Pakistan, and India (Cope 1982). This plant grows as a weed in dry and salty areas and is used as forage in underdeveloped countries. It is diploid (2n = 14) and has a relatively small genome (approximately 342 Mb). *A. littoralis* may serve as a rich genetic resource for the identification of new genes related to salt, drought, and heat tolerance (Zouari *et al.* 2007, Modarresi *et al.* 2012).

In the present study, CAT enzyme responses in the halophyte plant, *A. littoralis*, to NaCl stress were investigated and the CAT gene from this plant was isolated and characterized.

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Abbreviations: Car(s) – carotenoid(s); CAT – catalase; Chl – chlorophyll; DTT – dithiothreitol; EDTA – ethylenediaminetetraacetic acid; ORF – open reading frame; PMSF – phenyl methanesulfonyl fluoride; POD – peroxidase; PVP – polyvinylpyrrolidone; ROS – reactive oxygen species; RWC – relative water content.
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Materials and methods

Plants: *A. littoralis* was collected from Mian Kaleh natural habitats and cultured in the greenhouse of the Genetic and Agricultural Biotechnology Institute of Tabarestan. Equal clones were selected and their seeds were sterilized in 0.2% (w/v) NaClO for 1 min and grown under controlled conditions of 25–30°C, 70–90% relative humidity, 8/16 h night/day photoperiod, and photosynthetic active radiation of 250 μmol m^{-2} s^{-1}. They were hydroponically cultivated using a Hoagland nutrient solution (Hoagland and Arnon 1950). The nutrient solution was bubbled with air and changed once a week. Plants were grown for 30 d, and then the nutrient solution was supplemented with different NaCl concentrations (200, 400, and 600 mM). To avoid osmotic shock, salt concentrations were increased daily stepwise with 50 mM NaCl. A nutrient solution without NaCl was used as a control, and the evaporative conditions of the nutrient solution were controlled regularly. After 21-d treatment, fully expanded leaves were collected and used for chlorophyll (Chl) and relative water content (RWC) determination.

Pigment contents: Chl *a*, *b*, and total carotenoids (Cars) were determined spectrophotometrically according to the method of Porra (2002) at 665.2, 652.4, and 470 nm, respectively (Lichtenthaler and Buschmann 2001).

RWC: The fresh mass (FM) and dry mass (DM) of the harvested plants were used to determine RWC. It was estimated by recording the turgid mass (TM) of fresh shoot samples by keeping them in water overnight, then dried in a hot air oven till constant DM was achieved (Sairam and Srivastava 2002).

$$RWC = (FM - DM)/(TM - DM) \times 100$$

Protein extraction: Every 72 h, fully expanded leaves were collected, immediately frozen in liquid nitrogen, and stored at –80°C until use. Aliquots of frozen shoot material were ground to a fine powder with liquid nitrogen and extracted [500 mg(FM)/2 ml] at 4°C in a 100 mM Tris-HCl buffer (pH 8.0) containing 10 mM EDTA, 50 mM KCl, 20 mM $MgCl_2$, 0.5 mM PMSF, 1 mM DTT, 0.1% (v/v) Triton X-100, and 10% (w/w) PVP. The homogenate was centrifuged at 14,000 × *g* for 30 min at 4–8°C, and the supernatant was used for protein content determination and for measurement of CAT activities. Five replicates per treatment were used. Protein concentration was determined according to Bradford (1976) using the bovine serum albumin as a standard.

Enzyme assay: CAT activity was estimated as the decline in absorbance at 240 nm due to H_2O_2 consumption (Aebi 1984). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0) with 0.1 mM EDTA and 1 mM H_2O_2. The reaction was activated by adding 100 μl of the leaf crude extract. The reduction in absorbance was followed for 3 min; 1 unit decomposes 1 μmol of H_2O_2 per 1 min at 25°C and pH 7.

Specific amplification and cloning of CAT gene: Total RNA was isolated from leaves by using Trizol reagent (*Invitrogen*, USA) according to the producer's instructions. After treatment with DNAse I (*Roche*, USA), total RNA was used as the template for making cDNA by M-MULV reverse transcriptase (*Fermentas*, Lithuania) and reverse primer, which was designed based on the initial and terminal regions of CAT gene from other closely related plants, such as maize, barley, wheat, *etc*. Then cDNA was used as a template for following PCR. Gene-specific primers designed from Poaceae family plants, CATF (ATGGATCCCTGCAAGTTCCG), and CATR (TCACATGTTTGGCTTCACGTT) were used. PCR cycling conditions were as follows: 94°C for 5 min for initial denaturation; 35 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 2 min; and 72°C for 10 min for a final extension.

The amplified PCR products were loaded onto 1.5% TAE agarose gel and purified by DNA extraction kit (*Roche*, USA), then transformed into *Escherichia coli* by pTZ57R/T cloning vector (*Fermentas*, Lithuania). After recognization of transformed colonies by blue/white screening, PCR colony was performed and plasmids containing CAT gene were purified and sequenced.

Statistical and homology analysis: All analyses were done according to a completely randomized design at 5 replications. Data analyses were carried out using *SPSS version 18* and *Microsoft Excel* program software. Homology analyses and data base searching were carried out using the Genebank programs at http://www.ncbi.nlm.nih.gov/ and ExPASy tools at http://www.expasy.org/. Multiple sequence alignments and a phylogenetic tree were constructed with the program *Clustal X* at the http://www.ebi.ac.uk/ and *MEGA 5.0* software.

Results

Pigment contents: No changes occurred in Chl and total Car contents up to 400 mM, but they decreased compared with the control at 600 mM NaCl (Fig. 1).

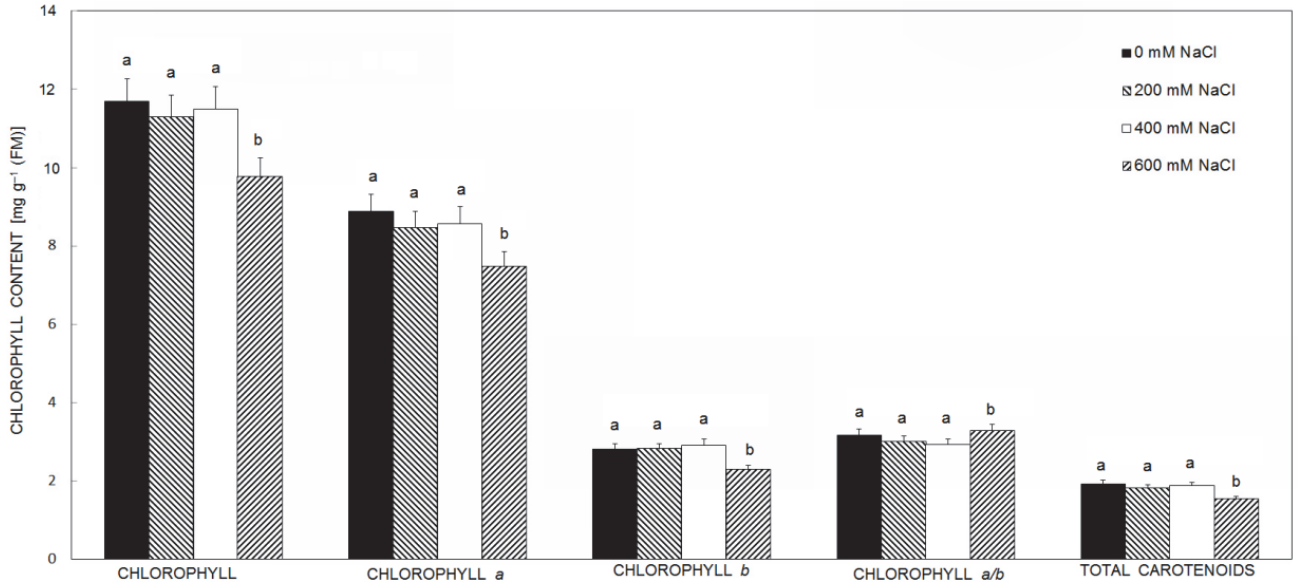

Fig. 1. Salt effects on chlorophylls and carotenoids of *Aeluropus littoralis*. *Different letters* in each column show significant difference at $p \leq 0.01$.

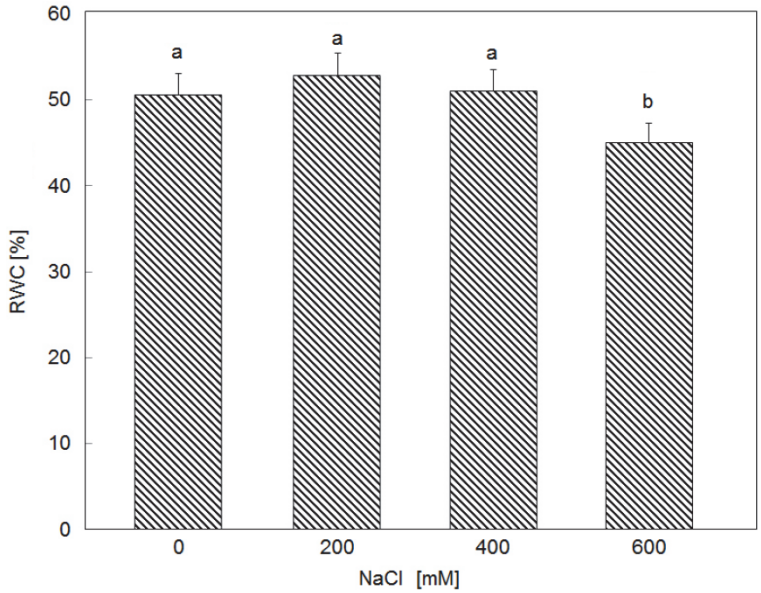

Fig. 2. Effect of salinity levels on relative water content (RWC) of *Aeluropus littoralis*. *Different letters* in each column show significant difference at $p \leq 0.01$.

RWC: There were no significant changes of RWC at 200 and 400 mM NaCl compared with the control, but RWC was significantly reduced at 600 mM NaCl (Fig. 2).

Enzyme assay: CAT activities at different NaCl concentrations are shown in Fig. 3. Similarly to SOD (data not shown), the maximum CAT activity was observed on the 12th day at 200 and 400 mM NaCl, while the maximal activity was observed on the 9th day at 600 mM NaCl (5.5, 7, and 5.5 fold, respectively, compared with the control). CAT and POD enzyme activities (data not shown) had the same trend. Additionally, the level of CAT activity was enhancing slightly during the first 3 d of stress, while POD activity increased highly during this period.

Amplification and cloning of CAT gene and bio-informatic analysis: The RT-PCR analysis indicated full length cDNA of CAT gene with a length of 1,485 bp (Fig. 4). The sequences of the gene showed that ORF was complete. This gene (*Alcat*) encoded 494 amino acids with a 56.925 kDa molecular mass including 6.52 isoelectric points. The novel gene contained start and stop codon, which meant that it could be the complete and functional gene. The lack of secreted peptide sequences at the N-terminal were assumed due to the fact that these

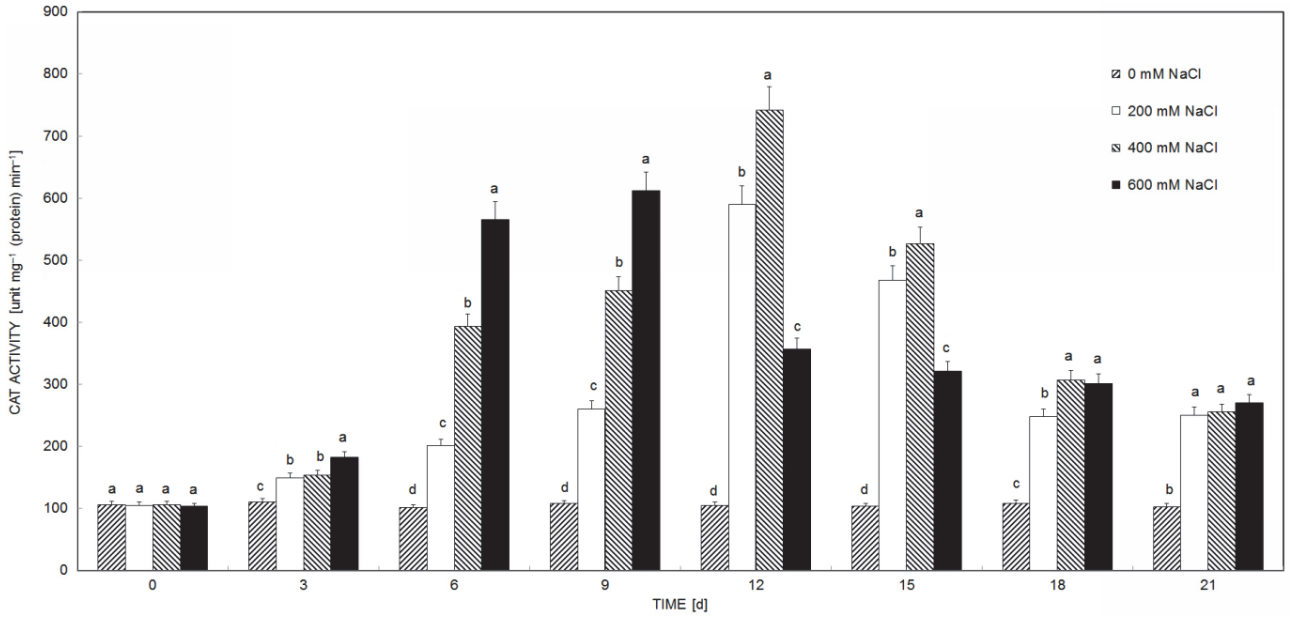

Fig. 3. The response of *Aeluropus littoralis* catalase to NaCl stress at different times (72 h periodically). *Different letters* in each column show significant difference at $p \leq 0.01$.

```
atggatccctgcaagttccggccgtcgagcagcttcgacacgaacgtctggaccacgaac
 M  D  P  C  K  F  R  P  S  S  S  F  D  T  N  V  W  T  T  N
gctggcgcccccgtctggaacaacaactccgcgctcaccgtcggacagcgaggccccatc
 A  G  A  P  V  W  N  N  N  S  A  L  T  V  G  Q  R  G  P  I
ctcctcgaggactatcatctgattgaaaagcttgcccagtttgacagggagcgtatccct
 L  L  E  D  Y  H  L  I  E  K  L  A  Q  F  D  R  E  R  I  P
gaacgtgttgtgcatgcccggggagcctgcgccaagggcttctttgaggtgacccacgat
 E  R  V  V  H  A  R  G  A  C  A  K  G  F  F  E  V  T  H  D
atgacgtcgctgacgtgcgccgacttcctgcggtcccccgggacgcggacgccggtgatc
 M  T  S  L  T  C  A  D  F  L  R  S  P  G  T  R  T  P  V  I
gtgcggttctcgacggtgatccacgagcgcgggagccccgagacgatccgggacccgcgc
 V  R  F  S  T  V  I  H  E  R  G  S  P  E  T  I  R  D  P  R
gggttcgccgtcaagttctacacgcgggagggcaactgggacctgctgggcaacaacttc
 G  F  A  V  K  F  Y  T  R  E  G  N  W  D  L  L  G  N  N  F
cccgtcttcttcatccgcgacggcatcaagttccccgacgtgatccacgcgttcaagccc
 P  V  F  F  I  R  D  G  I  K  F  P  D  V  I  H  A  F  K  P
aacccgaggtcgcacgtgcaggagtactggagggtgttcgactttctgtcgcaccacccg
 N  P  R  S  H  V  Q  E  Y  W  R  V  F  D  F  L  S  H  H  P
gagagcctgcacaccttcttcttcctcttcgacgacgtgggcgtgccgaccgactaccgc
 E  S  L  H  T  F  F  F  L  F  D  D  V  G  V  P  T  D  Y  R
cacatggaagggttcggcgtcaacacctacaccttcgtcaacgccgccggcaaggcgcac
 H  M  E  G  F  G  V  N  T  Y  T  F  V  N  A  A  G  K  A  H
tacgtcaagttccactggaagcccagctgcggggtgcggtccatcctcaccgacgaggag
 Y  V  K  F  H  W  K  P  S  C  G  V  R  S  I  L  T  D  E  E
gcggcgctcgtcgggggcggaaccacagccacgcgacgcaggacctctacgactccatc
 A  A  L  V  G  G  R  N  H  S  H  A  T  Q  D  L  Y  D  S  I
gcagcaggaaacttcccggagtggaaactcttcgtgcaggtgatggaccctgacacggag
 A  A  G  N  F  P  E  W  K  L  F  V  Q  V  M  D  P  D  T  E
gagcggtacgacttcgacccgctggacgacaccaagacctggcccgaggatctgctgccg
 E  R  Y  D  F  D  P  L  D  D  T  K  T  W  P  E  D  L  L  P
ttgcaacccgtcggaaggctcgtcctggaccggaacgtggacaacttcttcaacgagaac
 L  Q  P  V  G  R  L  V  L  D  R  N  V  D  N  F  F  N  E  N
gagcagctggcgttcggcccggggctggtcgtcccgggcatctactactccgacgacaag
 E  Q  L  A  F  G  P  G  L  V  V  P  G  I  Y  Y  S  D  D  K
atgctgcagtgccgcgtgttccgctacgccgacacgcagcgctacaggctcggccccaac
 M  L  Q  C  R  V  F  A  Y  A  D  T  Q  R  Y  R  L  G  P  N
tacctgatgctccccgtgaacgcgcccaagtgcgcgcaccacaacaaccactacgacggc
 Y  L  M  L  P  V  N  A  P  K  C  A  H  H  N  N  H  Y  D  G
gccatgaacttcatgcaccgcgacgaggaggtcgactacttcccctcccggcacgcgccg
 A  M  N  F  M  H  R  D  E  E  V  D  Y  F  P  S  R  H  A  P
ctgcgccacgccggcgcgccgacgccggtgcccgccaggccggtggccggcaagagggag
 L  R  H  A  G  A  P  T  P  V  P  A  R  P  V  A  G  K  R  E
aagacgaccatccggaagcagaacgacttcaagcagccgggggacaggtaccgctcatgg
 K  T  T  I  R  K  Q  N  D  F  K  Q  P  G  D  R  Y  R  S  W
gatcccgacaggcaggagcgcttcgtgcgccgcttcgccgactcgctcgtccaccacaag
 D  P  D  R  Q  E  R  F  V  R  R  F  A  D  S  L  V  H  H  K
gtcagccaggagctcagagcaatctggatcgactacctctcacagtgcgacacgtcgctt
 V  S  Q  E  L  R  A  I  W  I  D  Y  L  S  Q  C  D  T  S  L
ggatcaaagattgcggaccgcctcaacgtgaagccaaacatgtga
 G  S  K  I  A  D  R  L  N  V  K  P  N  M  -
```

Fig. 4. Nucleotide sequence of the cDNA encoding *Alcat* and amino acid sequence as one letter code below it.

genes most probably correspond with proteins located in cytosol. Our analysis indicated that *Alcat*-predicted protein contained conserved active site residues, such as H65, H79, S104, and N138 (Reilly *et al.* 2001). Primary structure analysis using *Protparam* (*ExPASy-Protparam* tools) indicated that the total number of negatively charged residues (Asp + Glu) was 64, while the total number of positively charged residues (Arg + Lys) was 59. *InterProScan* and *PROSITE* programs were used to find the heme-binding site and core domains. 9 amino acids at position 345–353 were found to be involved at the CAT heme-binding site. Immune-responsive domains existed in position 424–489 and CAT core domain was detected in 16–369 amino acids position (Fig. 5). CAT proximal heme ligand signature has a consensus pattern, R-[LIVMFST AN]-F-[GASTNP]-Y-x-D-[AST]-[QEH] in all species. In this pattern, Y is the proximal heme-binding ligand. *A. littoralis* had VFAYADTQ as its heme ligand pattern. Prediction of transmembrane helices and topology of proteins (available at http://www.enzim.hu/hmmtop/) indicated that our deduced amino acid sequence did not have any transmembrane helices, thus, it is not used as a membrane protein in nucleus or cytosol. The *NCBI Blast* program was used to determinate homology of CAT gene from *A. littoralis* and other species. Our data showed that *Alcat* had greater identity (90%) and similarity (94%) with *Z. mays* CAT gene (Fig. 7). *O. sativa, T. aestivum*, and *H. vulgare* had also high homology with 90, 87, and 86%, respectively. All of these plants are from the Poaceae family. The phylogenetic analysis of the amino acid sequence of *Alcat* compared with CATs of other plants indicated that this gene was closely related to *Z. mays* CAT gene (Fig. 7). The *SOPMA* program was used to investigate secondary

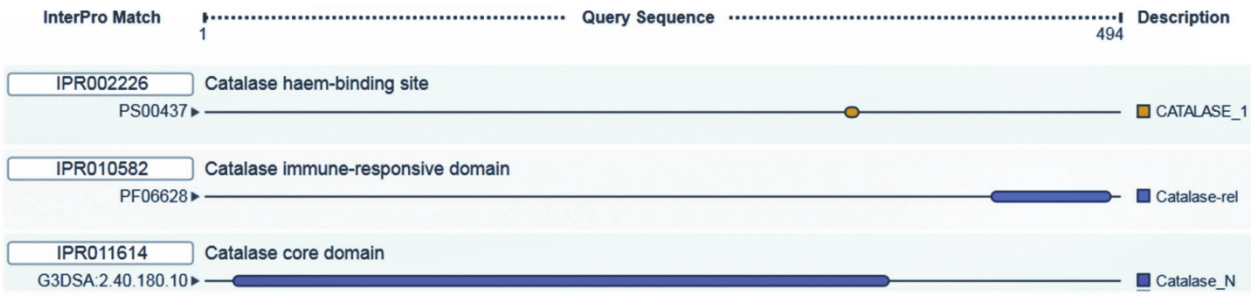

Fig. 5. Amino acid residues involved in haem-binding site, immune-responsive domain and core domain detected by *InterProScan* program.

```
Setaria italica          --MDPTKFRPSSSHDTTVTTTNAGAPVWNDNEALTVGPRGPILLEDYHLIEKVAHFARER 58
Zea maize                MTMDPTKFRPSSSHDTTVTTTNAGAPVWNDNEALTVGPRGPILLEDYHLIEKVAHFARER 60
Aeluropus littoralis     --MDPCKFRPSSSFDTNVWTTNAGAPVWNNNSALTVGQRGPILLEDYHLIEKLAQFDRER 58
Triticum aestivum        --MDPCKFRPSSSFDTKTTTTNAGQPVWNDNEALTVGPRGPILLEDYHLLEKIAHFARER 58
Oryza sativa             --MDPCKFRPSSSFDTKTTTTNAGAPVWNDNEALTVGPRGPILLEDYHLIEKVAHFARER 58
                           *** *******.**.. ***** ****:*.***** ***********:**:*:* ***

Setaria italica          IPERVVHARGASAKGFFECTHDVTSLTCADFLRSPGTRTPVIVRFSTVIHERGSPETIRD 118
Zea maize                IPERVVHARGASAKGFFECTHDVTSLTCADFLRAPGVRTPVIVRFSTVIHERGSPETIRD 120
Aeluropus littoralis     IPERVVHARGACAKGFFEVTHDMTSLTCADFLRSPGTRTPVIVRFSTVIHERGSPETIRD 118
Triticum aestivum        IPERVVHARGASAKGFFECTHDVTGLTCADFLRAPGARTPVIVRFSTVIHERGSPETIRD 118
Oryza sativa             IPERVVHARGASAKGFFECTHDVTDITCADFLRSPGAQTPVIVRFSTVIHERGSPETIRD 118
                         ***********.****** ***:*.:*******:**.:********************

Setaria italica          PRGFAVKFYTREGNWDLLGNNFPVFFIRDGIKFPDVIHAFKPNPRSHVQEYWRVFDFLSH 178
Zea maize                PRGFAVKFYTREGNWDLLGNNFPVFFIRDGIKFPDVIHAFKPNPRSHVQEYWRVFDFLSH 180
Aeluropus littoralis     PRGFAVKFYTREGNWDLLGNNFPVFFIRDGIKFPDVIHAFKPNPRSHVQEYWRVFDFLSH 178
Triticum aestivum        PRGFAVKFYTREGNWDLLGNNFPVFFIRDGIKFPDVIHAFKPNPKSHVQEYWRVFDFLSH 178
Oryza sativa             PRGFAVKFYTREGNWDLLGNNFPVFFIRDGIKFPDVIHAFKPNPRSHVQEYWRVFDFLSH 178
                         ******************************************:***************

Setaria italica          HPESLHTFFFLFDDVGVPTDYRHMEGFGVNTYTFVNAAGKAHYVKFHWKPTCGVRCILTD 238
Zea maize                LPESLHTFFFLFDDVGVPSDYRHMEGFGVNTYTFVSAAGKAQYVKFHWKPTCGVRCILTD 240
Aeluropus littoralis     HPESLHTFFFLFDDVGVPTDYRHMEGFGVNTYTFVNAAGKAHYVKFHWKPSCGVRSILTD 238
Triticum aestivum        HPESLHTFFFLFDDVGIPTDYRHMDGFGVNTYTFVSRAGKSHYVKFHWRPTCGVSCLM-D 237
Oryza sativa             HPESLHTFFFLFDDVGIPTDYRHMDGFGVNTYTFVTRDAKARYVKFHWKPTCGVSCLM-D 237
                          ***************:*:*****:**********.  .*::******:*:*** .:: *

Setaria italica          EEAALVGGRNHSHATQDLYDSIATGTFPEWKLFVQVMDPDTEEQYDFDPLDDTKTWPEDL 298
Zea maize                EEAALVGGRNHSHATQDLYDSIAAGSFPEWTLYVQVMDPDTEEQYDFDPLDDTKTWPEDL 300
Aeluropus littoralis     EEAALVGGRNHSHATQDLYDSIAAGNFPEWKLFVQVMDPDTEERYDFDPLDDTKTWPEDL 298
Triticum aestivum        DEATLVGGKNHSHATQDLYDSIDAGNFPEWKLFVQVIDPDDEDRFDFDPLDDTKTWPEDL 297
Oryza sativa             DEATLVGGKNHSHATQDLYDSIAAGNFPEWKLFVQVIDPEEEERFDFDPLDDTKTWPEDE 297
                         :**:****:************* :*.****.*:***:**: *:::**************

Setaria italica          LPLQPVGRLVLDRNVDNFFNENEQLAFGPGLVVPGIYYSDDKMLQCRVFAYADTQRYRLG 358
Zea maize                LPLRPVGRLVLDRNVDNFFNENEQLAFGPGLVVPGIYYSDDKMLQCRVFAYADTQRYRLG 360
Aeluropus littoralis     LPLQPVGRLVLDRNVDNFFNENEQLAFGPGLVVPGIYYSDDKMLQCRVFAYADTQRYRLG 358
Triticum aestivum        VPLQPVGRLVLDRNVDNFFNENEQLAFGPGLVVPGIYYSDDKMLQCRVFAYADTQRYRLG 357
Oryza sativa             VPLRPVGRLVLNRNVDNFFNENEQLAFGPGLVVPGIYYSDDKMLQCRVFAYADTQRYRLG 357
                         :**:*******:************************************************

Setaria italica          PNYLMLPVNAPRCAHHNNHYDGAMNFMHRDEEVDYYPSRHAPLRQ-APP-VPMPARPVVG 416
Zea maize                PNYLMLPVNAPRCAHHNNHYDGAMNFMHRDEEVDYYPSRHAPLRQAAPP-TPLPPRPVAG 419
Aeluropus littoralis     PNYLMLPVNAPKCAHHNNHYDGAMNFMHRDEEVDYFPSRHAPLRHAGAP-TPVPARPVAG 417
Triticum aestivum        PNYLMLPVNAPKCGFKNNHYDGAMNFMHRDEEVDYYPSRHAPLRHAEPASFPVPTRPVVG 417
Oryza sativa             PNYLMLPVNAPKCAHHNNHYDGAMNFMHRDEEVDYYPSRHAPLRHAPPT--PITPRPVVG 415
                         ***********:*..:*******************:********:  ..  *:..***.*

Setaria italica          RREKATIRKPNDFKQPGERYRSWDADRQERFVRRFADSLGHPKVSQELRSIWIDLLSKVG 476
Zea maize                RREKATIRKPNDFKQPGERYRSWDADRQDRFVRRFADSLGHPKVSQELRSIWIDLLAKCD 479
Aeluropus littoralis     KREKTTIRKQNDFKQPGDRYRSWDPDRQERFVRRFADSLVHHKVSQELRAIWIDYLSQCD 477
Triticum aestivum        KREKTRIKKENDFVQPGERYRSWAPDRQDRFVRRFADALAHPKVSHELRVIWIDFLSKCD 477
Oryza sativa             RRQKATIRKQNDFKQPGERYRSWAPDRQERFIRRFAGELAHPKVSPELRAIWVNYLSQCD 475
                         :*:*: *:* *** ***:***** .***:**:****. * * *** *** **:: *:: .

Setaria italica          NNLLAIIFFSDLIDRSYHTDDY 498
Zea maize                ASLGMKIATRLNMKANM----- 496
Aeluropus littoralis     TSLGSKIADRLNVKPNM----- 494
Triticum aestivum        KSCGMKVANRLNVKPSM----- 494
Oryza sativa             ESLGVKIANRLNVKPSM----- 492
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Fig. 6. Multiple alignment of *Alcat* (ADQ28492.1) amino acid sequence with CAT amino acid sequences from other plants *Elaeis guineensis* (ACF06566.1), *Musa acuminata* (ABV55108.1), *Triticum aestivum* (ADY02963.1), and *Hordeum vulgare* (P55308.1). Sequences are aligned by the program *CLUSTAL X*. * – identical amino acid residues, **:** – amino acids with high similarity, **·** – amino acids with low similarity, and **–** – gaps.

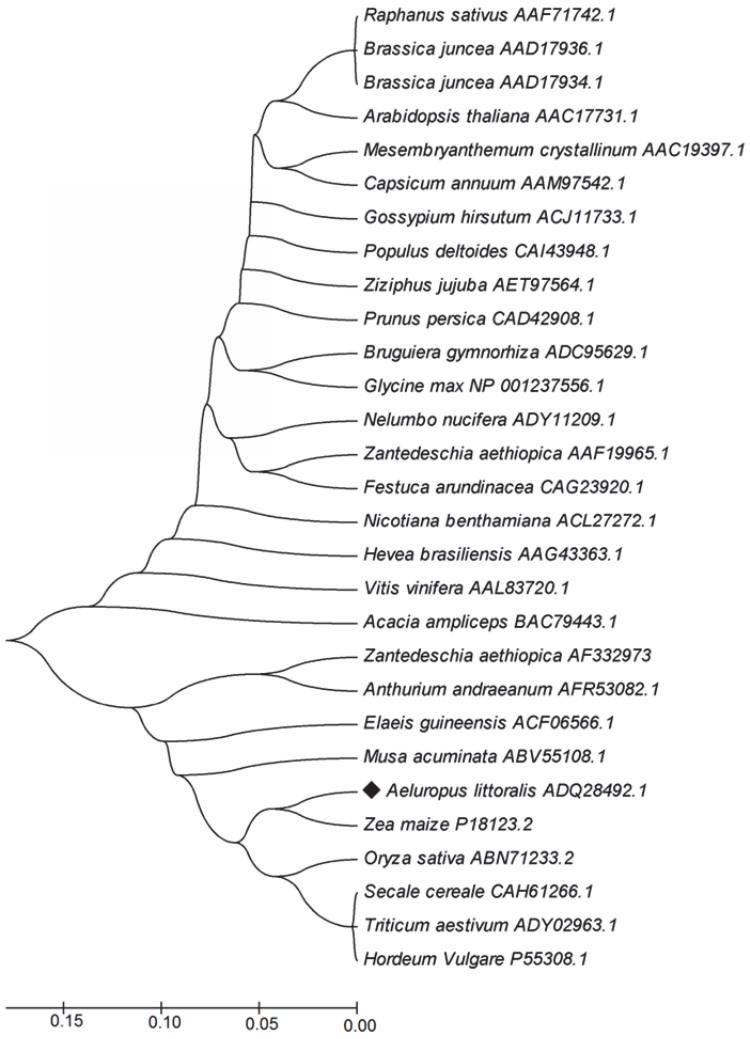

Fig. 7. Phylogenetic tree depicting the evolutionary relationship among catalase from different plants.

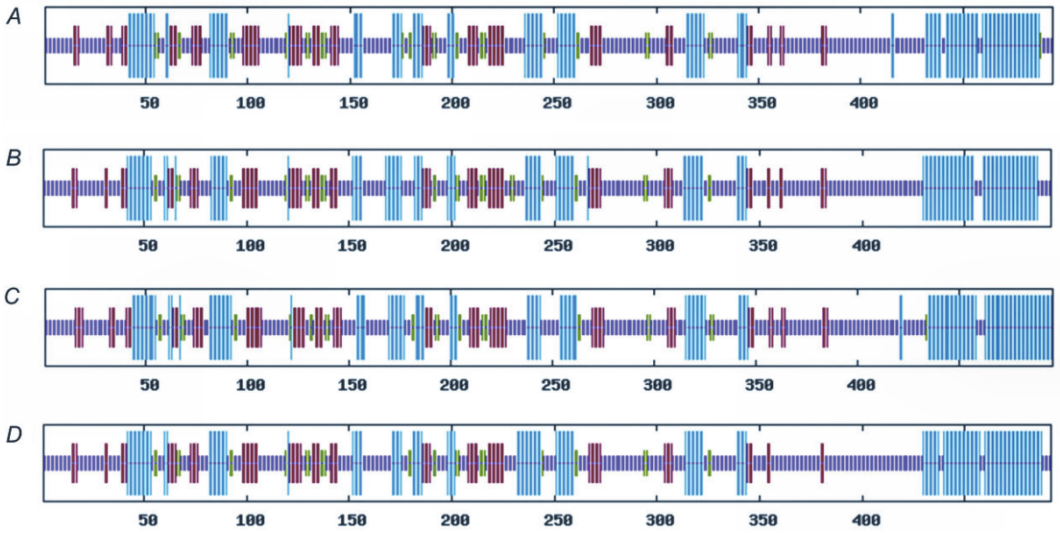

Fig. 8. Comparison of the CAT genes secondary structure of (*A*) *Aeluropus littoralis* (ADQ28492.1), (*B*) *Oryza sativa* (ABN71233.2), (*C*), *Zea mays* (P18123.2), and (*D*) *Vitis vinifera* (AAL83720.1). The helix, sheet, turn, and coil are investigated by lines in order from the tallest to the shortest.

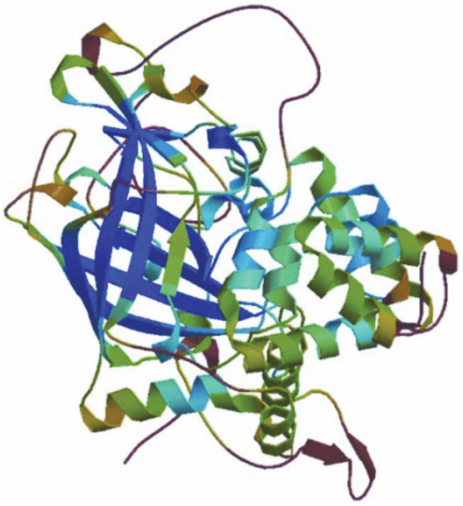

Fig. 9. The 3D structure of *Aeluropus littoralis* catalase protein predicted by *SWISS-MODEL* software.

structure analysis. Results revealed that *Alcat* consists of 130 alpha helices, 30 beta turns, 77 extended strands, and 257 random coils. These results are similar to those of the secondary structure analysis of *O. sativa* with 130 α-helices, 28 β turns, 74 extended strands, and 260 random coils. Also, *Secale cereale* has 131 α-helices, 32 β turns, 73 extended strands, and 258 random coils (Fig. 8). *A. littoralis* did not have any β bridge in its CAT secondary structure as other similar plants. It seems that salt-tolerant species, such as *A. littoralis* and *Setaria italica* (foxtail millet), have more extended strands. It is possible that extended strands are effective in tolerance to unfavourable environmental conditions. The 3D structure of *Alcat* was predicted by the *Swiss-Model* program (Fig. 9). This program used 1m7sA from CAT crystal structure of *Pseudomonas syringae* as a template. The root mean square deviation between *Alcat* and the template '1m7sA' was 1.80 Å over 479 aligned residues.

Discussion

A. littoralis is a halophyte C_4 grass belonging to the Poaceae family (Zouari *et al.* 2007). The primary results of this study indicated that this halophyte plant needed almost 150–250 mM NaCl for its optimal growth. Under high NaCl concentration (600 mM), plant growth decreased and plant internodes and leaf surface were reduced compared with control plants (data not shown). Previously, Khan *et al.* (2001) and Sobhanian *et al.* (2010) showed that growth of two halophyte plants, *Salicornia rubra* and *Aeluropus lagopoides,* increased at 200 and 150 mM NaCl concentration, respectively. It seems that halophyte plants use moderate salinity for osmotic adjustments, maintenance of turgor and cell water content (Flowers and Colmer 2008).

This result indicated that leaf photochemistry in *A. littoralis* was well protected under salt stress, as it was also reported on salt-stressed barley (Seckin *et al.* 2010) and maize cultivars (Shabala *et al.* 1998). Previously, Barhoumi *et al.* (2007) reported that Chl (*a* + *b*) and Car content were not significantly influenced in *A. littoralis* by salt treatment until 400 mM NaCl. The decrease in Chl *a* and Chl *b* might be due to either the increased activity of the Chl degrading enzyme, chlorophyllase (Rao and Rao 1981), or the destruction of the chloroplast structure and the instability of pigment-protein complexes in 600 mM NaCl (Noreen and Ashraf 2009). Also at 600 mM NaCl stress, the Chl *a*/*b* ratio increased. A damage of mesophyll cell chloroplast was one of reasons for higher Chl *a*/*b* ratio in *A. littoralis* leaves.

RWC stable at 200 and 400 mM NaCl showed that this halophyte plant was highly tolerant to salt stress. Lee *et al.* (2005) have already reported that RWC of seven moderately or highly tolerant seashore paspalums was statistically similar to that of the control until 300 mM NaCl. It was directly reflective of the water status of plants and its decrease suggested that the salinity caused water deficit in the plants. The negative effect on plant water relations was induced by accumulation of soluble salts, which slowed down the uptake of water and nutrients, causing osmotic stress effects and toxicity (Yang *et al.* 2009), despite the fact that RWC of some other halophytes, such as *Sauda salsa* (Lu *et al.* 2002) and *Atriplex nummularia,* was not changed or enhanced under salt-stress conditions (Silveira *et al.* 2009). According to Mohsenzadeh *et al.* (2006), a reduction in RWC of up to 5% produced no negative impact on plant health and growth.

CAT enzyme activity increased under NaCl stressful condition. CAT has a significant role in the plant defense system against oxidative stress. It is produced in peroxisomes and glyoxysomes. This enzyme catalyzes a redox reaction, in which dismutation of H_2O_2 gives rise to water and oxygen (Ali and Alqurainy 2006, Ashraf 2009). Increases in CAT activity after salt exposure have been mentioned by Pérez-López *et al.* (2010) and Yang *et al.* (2009), however, a reduction of CAT activity has been reported in certain plant species exposed to numerous environmental stresses, such as salinity (Parida and Das 2005) and drought (Zhang and Kirkham 1994). Khosravinejad *et al.* (2008) observed a rapid and continued increase in CAT activity of salt-tolerant barley cultivars under salt stress. In cotton, significantly higher constitutive concentrations of CAT and POD were found in salt-tolerant lines than in salt-sensitive lines, while the activities of these enzymes remained unchanged or decreased in salt-sensitive cultivars (Gossett *et al.* 1996).

The CAT enzyme can play an important role in tolerance to salinity in many halophyte plants. These halophytes belong to the Poaceae family and they can be highly efficient genetic sources for tolerance of abiotic

stress for improving other species such as wheat, rice, and so on. To achieve this goal some antioxidant enzyme genes were isolated and cloned (Modarresi *et al.* 2012), such as SOD (HM107007), CAT (HQ389206), and APX (JF907687, JF819725). Further experiments for transformation of these genes to crop plants and analysis of the transformed plants responses under salt stress conditions are currently underway.

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