Identification and characterization of *CAT1* gene during drought stress in moth bean [*Vigna aconitifolia* (Jacq.) Marechal]

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Summary

Moth bean [*Vigna aconitifolia* (Jacq.) Marechal] is a drought-hardy orphan pulse crop. However, the information related to the identification and characterization of drought stress tolerance genes is very limited. Therefore, the present investigation was formulated to identify and characterized drought-tolerant gene(s) from moth bean. Five genes were selected from available expression studies of moth bean and their expression pattern was assessed during a time course experiment of drought stress in moth bean. During the time-course drought stress experiment in moth bean, the *catalase1* (*CAT1*) gene was exponentially expressed and up-regulated. Thus, *CAT1* gene of the moth bean was identified as a potential candidate gene and validated through Sanger's sequencing. The genomic sequence *CAT1* gene was named *VacoCAT1* and was further characterized using various bioinformatics tools. The *VacoCAT1* showed an incomplete ORF with a length of 213 bp, which encoded 71 amino acids. The coding sequence of *VacoCAT1* gene was shown as a single exon due to the incomplete nature of genomic sequences. The multiple sequence alignment of *VacoCAT1* revealed the highly conserved region at the 3' site of the gene as compared to *CAT1* gene of other crop species, including legumes. The phylogenetic analysis of *VacoCAT1* and *CAT1* genes of other crop species, including legumes, revealed three clusters. The cluster *VacoCAT1* gene showed proximity with *V. radiata CAT1* in cluster one of a phylogenetic tree. The identified and characterized *VacoCAT1* gene can be utilized as a genomic resource for enhancing drought tolerance in susceptible pulses and other crops.

JAE 2022, Vol 14
Received: 25 Oct. 2022
Accepted: 15 Nov. 2022
Published: 15 Dec. 2022
https://doi.org/10.53911/JAE.2022.14224

Associate Editor: Dr. S. M. Haldhar

Keywords: Bioinformatics analysis, *CAT1* gene, drought stress, gene expression, sequence validation

INTRODUCTION

Moth bean [*Vigna aconitifolia* (Jacq.) Marechal] is an annual legume crop which belongs to the subfamily Papilionaceae of Fabaceae. It indicates the wide social acceptance and geographic adaptation and is considered as the native pulse of India. It is, however, the most crucial pulse in arid zone perspective. During the extremity of drought stress, this is the only crop, cultivated for grain and fodder with minimum water, tillage and other agronomic inputs.[1] In order to combat the adverse effects of drought, it is very essential to develop water-deficit stress-tolerant genotypes in crop plants. To achieve this, a better understanding of the stress-induced responses and the inter-relationships of physiological and biochemical traits in drought tolerant crops needed to be studied. Since, moth bean is a drought-hardy pulse thus it can prove the usefulness of drought stress tolerance mechanisms.[2, 3, 4] Additionally, the moth bean is a native crop of hot and dry habitats of northern and western parts of India. In arid and semi-arid regions drought as abiotic stress, is one of the major factors limiting plant growth at various stages of their life. Water stress in plants reduces the water potential and turgor and increases the concentration of solutes in the cytosol. Consequently, cell enlargement, gas exchange, transpiration, plant nutrients uptake and transport are decreased.[5] A major effect of drought is a reduction in photosynthesis, which arises from a decrease in leaf expansion, impaired photosynthetic machinery, premature leaf senescence and associated reduction in food production.[6] Acclimation of plants to water deficit is the result of different events, which lead to adaptive changes in plant growth and physio-biochemical processes, such as changes in plant structure, growth rate, tissue osmotic potential and antioxidant defences.[7] It has been recognized that plants exhibit several adaptations to survive under stress conditions. Reduced leaf area, stomatal closure to prevent transpirational water loss, decreased stomatal conductance, limited internal CO_2 concentration, reduced photosynthesis are very vital.[8] Water-deficit stress tolerance is thus the result of coordination of

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physiological and biochemical alterations at the organ, cellular and molecular levels.[9] Although these physiological and biochemical mechanisms of the stress response are common to all plants, major differences exist in terms of strategies adopted by different crop species to cope with drought stress.[10] Drought-tolerant and sensitive varieties differed in their protein profiling with newer genes showing their expression in tolerant ones. Higher basal activity of enzymes and total soluble sugar during water deficit in drought-tolerant varieties was reported by Soni *et al.* (2011).[2]

Since the moth bean is known as stress hardy orphan pulse[11], however, expression studies to understand stress tolerant mechanism at the molecular level is very limited. Few studies have been brought to understand the drought tolerant mechanism in moth bean.[12, 2, 3, 4] However, molecular cloning and characterization of stress-responsive genes have not been reported in moth bean. Thus, in order to combat the adverse effects of drought, it is very essential to develop water-deficit stress-tolerant genotypes. Thus, a better understanding of the stress-induced responses and the inter-relationships of physiological and biochemical traits in drought tolerant crops such as moth bean can prove to be very useful. Therefore, few drought stress-responsive genes were profiled at expression level during drought stress in moth bean to identify the best drought-tolerant gene.

Materials and Methods

Plant material and gene-specific oligos

Seeds of moth bean cv. RMO-40 were collected from Agricultural Research Station (ARS), Swami Keshwanand Rajasthan Agricultural University, Bikaner. Five up-regulated genes from publicly available expression studies of moth bean (Table 1) were selected and genomic sequences pertaining to selected genes were identified in EST database of moth bean (https://www.ncbi.nlm.nih.gov/nuccore) and used for designing gene-specific primers using PrimerQuest software (https://www.idtdna.com/pages/tools/primerquest). The gene-specific primers were synthesized from GCC Biotech Pvt. Ltd, India.

Table 1: Selected genes and gene-specific oligos used in this study

Genes	Primer Sequence (5'-3')	Tm	Reference
CAT1	F – CTTCTGGAGGATTATCATCT	55 °C	Tiwari et al. (2018)[4]
	R – AAGGAGAACATGTGAAGGCT		
HSP70	F – ATGACAGTGTTGATTCCGAG	55 °C	Tiwari et al. (2018)[4]
	R – TCTTCATCCTCTGCCTTGTA		
HSP90	F – GACGATGTGAATCAACTGCT	55 °C	Tiwari et al. (2018)[4]
	R – CTATATAGGATCACCAGCA		
P5CS	F – GCAGATTAAGGAGCTGAACT	55 °C	Priya et al. (2015)[15]
	R – TGTCACATCTAGCTGCAGGA		
P450	F – GAGAAGACATTGGTGTATGTG	55 °C	Tiwari et al. (2018)[4]
	R – GAAGCATATCAGTATCTATGTC		
ACTIN	F – AGCCACTGGAATTCATGAAACGACA	55 °C	Rampuria et al. (2012)[13]
	R – TGCTGCTTGGTGCTAGAGCACTG		

Growing of plants and drought stress treatment

The seeds of RMO-40 were grown in 8-inch plastic pots pre-filled with a 1:1 ratio of field soil and perlite. The seedling was raised in a plant growth chamber (Cole-Parmer, India) by providing controlled environmental conditions (30 °C temperature, 60% relative humidity and 16/8 light-dark cycle). The seedlings were irrigated thrice a week with Hoagland solution (Hi-media, India). After 15 days, only one healthy seedling per pot was retained by up-rooting of other comparatively less vigoured seedlings. Two sets of seedlings were raised for control and drought stress treatment, respectively. For creating drought stress, fifteen days old seedlings were subjected to drought and heat stress by the withholding of water and at 35 °C, respectively. The control samples were maintained by irrigation with Hoagland solution parallelly (Fig 1). The leaf samples were collected from drought stress-treated seedlings along with the control sample (seedling without stress) at 0, 3, 6, 24 and 72 hours of post-stress. The experiment was conducted in three subsequent biological replication and the statistical significance of the replication was assessed using a student's t-test at p-value <0.05.

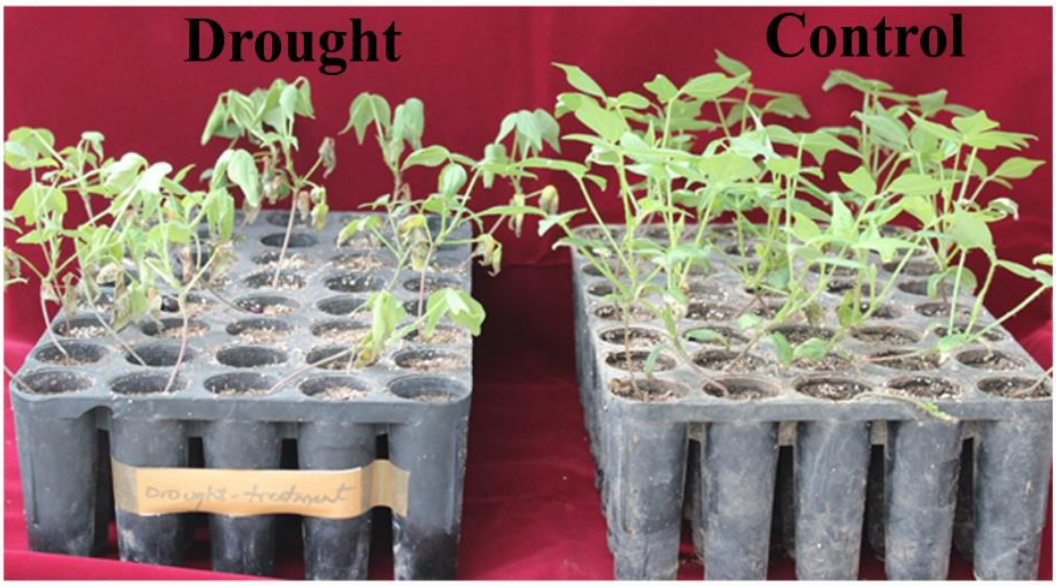

Fig 1: Planting material with drought stress and normal (control) conditions during drought-stress treatment.

RNA isolation and cDNA synthesis

The total RNA was isolated from 100 mg of moth bean leaf samples using an RNeasy plant mini kit (Qiagen, Germany) according to the manufacturer's instructions. The genomic contamination from total RNA was removed by treating the solution with RNase-free *DNaseI* (Invitrogen, USA). The quality and quantity of total RNA were assessed electrophoretically on 2% agarose gel and spectrophotometer (Shimadzu, Japan), respectively. The RNA quality was assured on the agarose gel by visualizing it under UV-transilluminator. The quantities of the RNA samples were determined at 260 nm absorbance and a ratio from 1.9 to 2.0 was considered as good quality RNA. The 3 µg of total RNA was used to reverse transcribe into cDNA as per the manufacturer's instructions (Takara Bio. Inc., Japan). The uniformity and quality of cDNA were assessed by amplification of *actin* gene of moth bean.

Semi-quantitative reverse transcriptase PCR analysis

The gene-specific primers were validated in drought stress-treated samples of moth bean using semi-quantitative reverse transcriptase PCR (semi-qRT PCR) analysis. The PCR reaction was performed in 20 µl reaction volume containing 10 µl of Dream Taq master mixture (Genetix Biotech Asia Pvt. Ltd., India), 1.0 µl of each forward and reverse primers (10 mM), 1 µl $MgCl_2$ (10 mM), 2 µl of a template (cDNA) and nuclease-free water (Hi-media, India) for volume make-up. The PCR was performed in a thermal cycler machine (Bio-Rad, USA) with the following thermos cycling conditions: one step of initial denaturation at 94 ºC for 5 min. followed by 28-30 cycles of denaturation at 94 ºC for 30 s, primer annealing at 55 ºC for 30 s and primer extension at 72 ºC for 2 min. followed by one step of final extension at 72 ºC for 10 min. The PCR products were visualized electrophoretically on 2.0% agarose gel and documented in gel documentation system (SynGene, USA). The house-keeping gene (*actin*) of moth bean was used as an internal control.[13]

Sequence characterization

The exponentially expressed *catalase* gene in terms of up-regulated manner during the time course experiment of drought stress was selected for Sanger's sequencing to determine the nucleotide sequences. The *catalase* gene was amplified on cDNA of moth bean. The PCR product was purified using QIAquick PCR purification kit (Qiagen, Germany) and validated through sequencing from GCC Biotech Pvt Ltd., India. The resultant nucleotide sequence of the catalase gene was validated through a homology search in NCBI Blast analysis (https://blast.ncbi.nlm.nih.gov/Blast) and the *catalase* gene of moth bean was nomenclature as *VacoCAT1* gene. The open reading frame was predicted from the resultant genomic sequence of *VacoCAT1* gene using ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/).

Gene structure and conserved motifs analysis

Full-length gene sequences and corresponding CDS of *catalase* gene were downloaded from the Legume Information System database of legume crops (https://www.legumeinfo.org/). The retrieved genomic information of *catalase* gene of legume crops was compared with *VacoCAT1* gene for prediction of exon-intron distribution using PIECE2 software (http://probes.pw.usda.gov/piece or http://aegilops.wheat.ucdavis.edu/piece). The conserved motifs in *VacoCAT1* were examined in MEME suite (https://meme-suite.org/meme/) with 20 numbers of motifs as parameters.

Multiple sequence alignment and phylogenetic analysis

For multiple sequence alignment analysis, nucleotide sequence encoded amino acid sequences of *VacoCAT1* were aligned with *catalase* genes of related legumes using

Clustal omega online tool (https://www.ebi.ac.uk/Tools/msa/clustalo). The amino acid sequence alignment was graphically represented by ESPript 3.0 online software (http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi). To reveal the phylogenetic relationship between the *VacoCAT1* and *catalase* genes of related legumes and *Arabidopsis,* multiple sequence alignment of amino acid sequences were established further and alignment was utilized for the phylogenetic tree construction using poison model and Neighbor-joining method with 1000 bootstrap replicates in MEGA 6.0 software (www.megasoftware.net).

Results and Discussion

Assessment of drought stress-responsive expression of moth bean genes

Moth bean has peculiar traits like a tap root system, the spreading nature of plants which make it stressful hardy legume. Moth bean possesses resistance to extreme temperature regimes.[3, 14] Our experiment conducted on moth bean during withholding of water and grown at 35 ºC has shown that these seedlings can efficiently live and perform their metabolic activity. Thus, owing to resistance against heat and drought stress, it is quite imperative to identify and characterize genes related to drought stress tolerance mechanisms at the molecular level. Thus, in the present investigation, the physiological adoption of the moth bean was tried to understand at the molecular level. It was aimed to achieve five reported up-regulated genes during drought and heat stress in moth bean.[4, 15] The expression of genes for abiotic stress responsiveness was carried out by various gene expression studies in legumes.[3, 13, 16] Similarly, in the present study, the expression characterization of selected genes was done by semi-qRT PCR analysis. This was done to select the gene which shows the exponential up-regulation during the time course experiment of drought in moth bean.

To validate the expression of five drought-tolerant genes of moth bean during drought stress, a semi-qRT-PCR analysis was done using gene-specific primers. The semi-qRT-PCR assay (Fig 2) revealed that the *CAT1* gene expressed exponentially in the up-regulated manner in a time course study from 0 to 72 h of drought stress. Exceptionally, this gene was observed down-regulated only at a single time point (24 h) as compared to the control. Such type of interruption in the exponential expression of *CAT1* gene was suggested that the stability of the encoded protein product to give tolerance against drought stress is up to 24 h or less. However, this gene was expressed at later time points (72 h) which also suggested the requirement of encoded protein against drought stress in moth bean. The *HSP70* gene was significantly up-regulated at 6 h; whereas, it was significantly down-regulated as compared to the control at 24 and 72 h of drought stress. The *HSP90* gene was down-regulated at all-time points of drought stress but it was significantly further down-regulated at 72 h. Similarly, the *P450* gene was down-regulated at 3 and 72 h of drought stress, whereas it was significantly up-regulated at 6 and 24 h of drought stress as compared to the control. This gene showed a circadian type of expression pattern during drought stress experiments in moth bean. The *P5CS* gene was not shown a significant expression in the time course experiment of drought stress in moth bean.

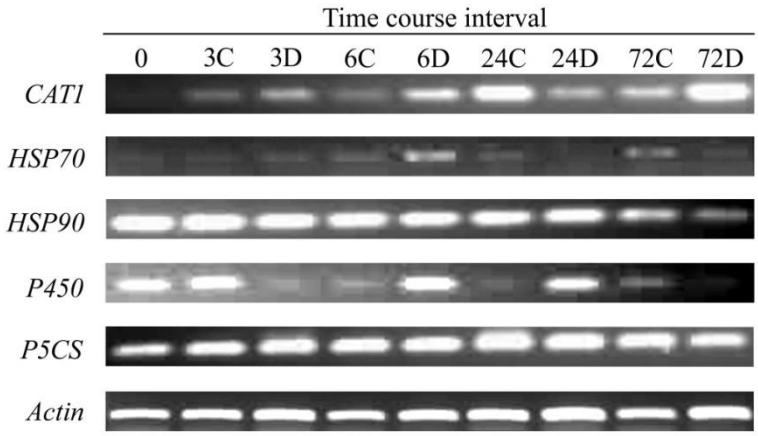

Fig 2: Expression profiling of drought stress tolerant genes during drought stress in moth bean. The alphabets C and D indicate control and drought stress, respectively.

Heat-shock proteins including HSP70, HSP90 also identified as heat stress tolerance genes in crop plant.[11, 12, 17, 18] In the present study, the *HSP70* and *HSP90* genes were also analyzed during drought stress conditions in moth bean. However, *HSP70* and *HSP90* genes were showed circadian type of gene expression with down-regulation. Similarly, the *P450* gene was showed up-regulation during drought stress in moth bean gene expression studies.[4] However, it was expressed contradictorily in the present investigation. It also showed the circadian type of gene expression with down-regulation. The *P5CS* gene also showed potential expression during stress conditions in moth bean.[15] In the

present study, *P5CS* gene was significantly not expressed during drought stress in moth bean. The contradictory issues related to the expression of *HSP70*, *HSP90*, *P450* and *P5CS* genes during abiotic stress conditions in moth bean could be due to the different experimental conditions of reported studies and present investigation.

The *catalase* gene showed up-regulation in the gene expression study of moth bean[4] and other various crop plants.[19, 20] Similarly, the *catalase* gene was also found to be the most up-regulated gene as compared to the other four genes during drought stress conations in RMO-40 in our study. Thus, *catalase* gene was selected as a potential drought-tolerant candidate gene of moth bean for sequencing and further characterization by bioinformatics means.

Selection and sequencing of potential drought tolerant gene, *VacoCAT1*

Based on the expression pattern during drought stress experiment in moth bean, the selected and characterized five drought tolerant genes were categorized into three main groups such as (i) the exponentially up-regulated with sustainable expression – *CAT1* gene; (ii) genes with circadian and down-regulation in expression – *HSP70*, *HSP90* and *P450* gene; and (iii) significantly not expressive gene – *P5CS* gene. Thus, based on the sustainable expression during the time course experiment of drought stress in moth bean, the *CAT1* gene was considered as a potential candidate as the drought-tolerant gene of moth bean. Identifying genes of interest is immensely needed in sequence analysis and functional annotation with existing known genes.[21] Therefore, the *CAT1* gene was amplified at cDNA level and it was validated through Sanger's sequencing. A good-quality nucleotide sequence of the partially amplified *CAT1* gene of moth bean was obtained from the Sanger's sequencing and the gene was named as *VacoCAT1* gene. The *VacoCAT1* gene showed high sequence similarities with *catalase1* gene of *V. radiata*.

Structural analysis of *CAT1* gene

To predict the structure of *VacoCAT1* gene of moth bean in terms of exon-intron distribution, number and length of open reading frame (ORF), peptide length, etc., the nucleotide sequences obtained from Sanger's sequencing were analyzed using various online softwares. The exon-intron distribution of genes is revealing structural organization and evolutionary events of the genes.[22] Thus, for exon-intron distribution, the *VacoCAT1* gene was compared with *CAT1* gene of *Vigna radiata* in Gene Structure Display Server 2.0 software. The analysis revealed only one exon of around 650 bp length in *VacoCAT1* gene. No introns were predicted by the software due to the incomplete nature (partial) of nucleotide sequences of *VacoCAT1* gene (Fig 3a). The ORF finder revealed the four ORF in *VacoCAT1* gene. Out of four ORF predicted, the ORF1 was found to be the longest (213 bp) which encoded 71 amino acids (Fig 3b).

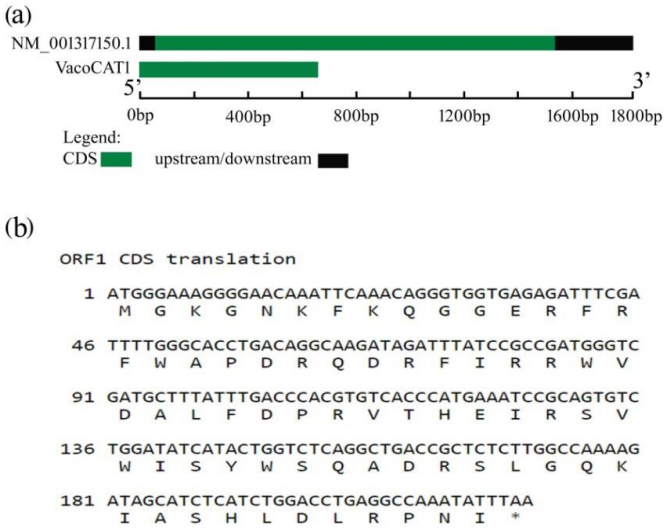

Fig 3: Predicted gene structure (a) and ORF characterization (b) of *VacoCAT1* gene

Multiple sequences alignment and prediction of conserved motifs

The multiple sequence alignments in amino acid sequences of genes reveal the sequence similarity and conservation among the sequences. The multiple sequence alignment was done in amino acid sequences of *CAT* genes of various crop plants including legume crops.[23, 24, 25] Similarly, the nucleotide sequence of *CAT1* gene of moth bean was aligned and compared with *CAT1* genes of *V. radiata*, *V. angularis*, *V. unguiculata* and *Arabidopsis thaliana*. The MSA analysis revealed a significant sequence homology with conservation among the nucleotide sequences of *CAT1* gene of moth bean along with *CAT1* gene of *V. radiata*, *V. angularis*, *V. unguiculata* and *A. thaliana* (Fig 4). The sequence similarity analysis showed close proximity of *CAT1* gene of moth bean with *CAT1* gene of *V. radiata*. The analysis also revealed the highly conserved region among the polypeptide of *CAT1* genes was amino acids at 3' region of the gene. For the prediction of the conserved motif present in *CAT1* gene of the moth bean, the peptide sequence of *CAT1* gene was analyzed in MEME suite software. The analysis revealed 6 motifs presented in the *VacoCAT1* gene as compared to the *CAT1* gene of *V. radiata*. The discrepancy in the conserved motif was found due to the partial nature of gene sequences.

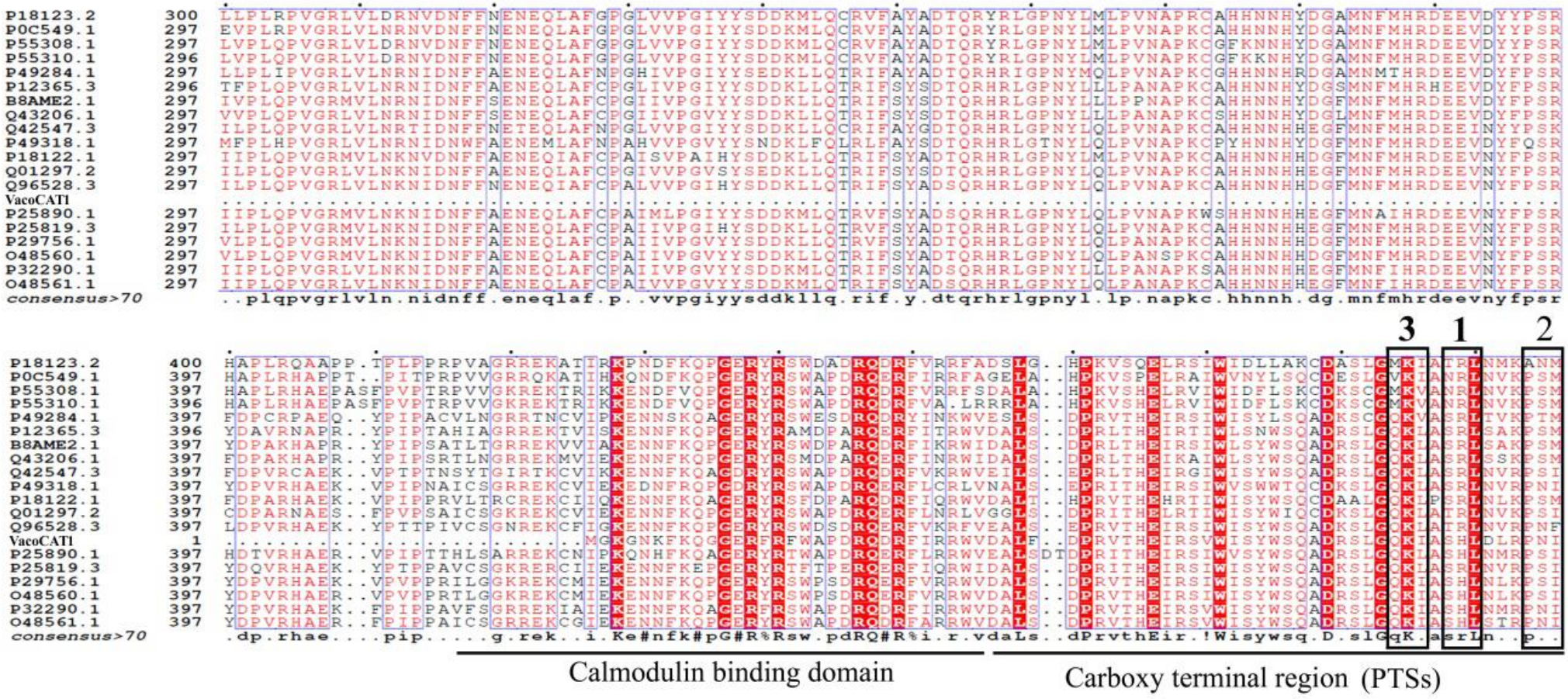

Fig 4: Multiple sequence alignment analysis of *VacoCAT1* gene with *catalase* gene of other plant species

Phylogenetic analysis

The nucleotide sequence of *VacoCAT1* gene was compared along with *CAT1* genes of related legume and *A. thaliana* during phylogenetic analysis. The phylogenetic tree revealed 3 clusters of *CAT1* genes (Fig. 5). The cluster I was further divided into two sub-groups representing one group with *CAT1* genes of legume crops and another with *A. thaliana, Z. mays*, wheat and rice. The phylogenetic analysis also showed the closed proximity of *VacoCAT1* gene with *CAT1* gene of *V. radiata* as shown in the MSA analysis. The phylogenetic analysis in this study showed the *CAT* genes of legumes in the same phylogenetic clade. The analysis suggested that the *CAT1* gene is evolutionarily conserved in legume crops. The phylogenetic conservation of *catalase* genes was also found in many organisms including crop plants.[26, 27, 28]

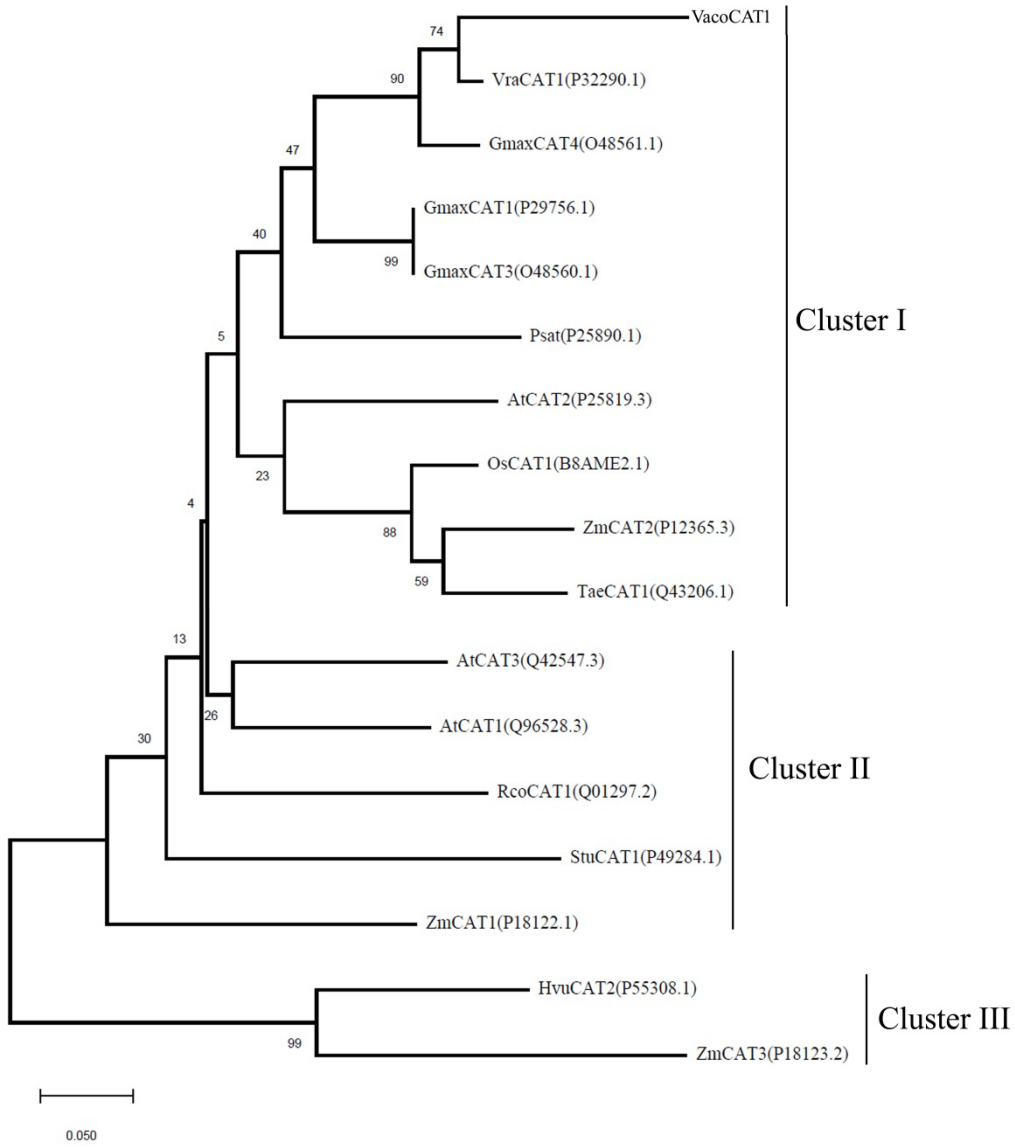

Fig 5: Phylogenetic analysis of *VacoCAT1* gene with *catalase* gene of other plant species

Conclusion

In conclusion, the five drought responsive genes were expressed differentially during drought stress in moth bean. Out of five expressed genes, *catalase1* gene was expressed exponentially in up-regulated manner as compared to control. Thus, based on up-regulation during drought conditions in moth bean, the *catalase1* gene was selected as drought tolerant candidate gene of moth bean. The *catalase1* gene of moth bean was validated through sequencing and characterized based on various bioinformatics tools and software. The *VacoCAT1* gene showed significant sequence homology with *CAT1* gene of *V. radiata* and proximity with *CAT1* gene of legume crops during phylogenetic analysis. The molecular cloning of *VacoCAT1* gene at the full-length level and functional validation of the gene is the future area of this study. The *VacoCAT1* gene can be utilized as a heterologous candidate gene for the development of abiotic stress tolerance in legume crops.

Acknowledgement

The authors are thankful to the Vice-chancellor, SKRAU, Bikaner and the Director, ICAR-CIAH, Bikaner for providing financial support to complete this study.

Declaration of interests

The authors have no conflict of interest to declare.

Data sharing

All relevant data are within the manuscript.

References

1 Bhati TK, Shalander K, Amare H & Whitbread AM. 2017. Assessment of agricultural technologies for dryland systems in South Asia. Monograph. ICRISAT, Patancheru, Hyderabad, pp - 68.

2 Soni P, Rizwan M, Bhatt KV, Mohapatra T & Singh G. 2011. *In-vitro* response of *Vigna aconitifolia* to drought stress induced by PEG–6000. *Journal of Stress Physiology and Biochemistry,* 7:108-121.

3 Tiwari B, Kalim S, Bangar P, Kumar S, Kumari R, Barman P & Bhat, K.V. (2017). Expression analysis in response to heat stress in moth bean (*Vigna aconitifolia* (Jacq.) Marechal). *Electronic Journal of Plant Breeding* 8: 1293-1297.

4 Tiwari B, Kalim S, Tyagi N, Kumari R, Bangar P, Barman P, Kumar S, Gaikwad A & Bhat KV. 2018. Identification of genes associated with stress tolerance in moth bean [*Vigna aconitifolia* (Jacq.) Marechal], a stress-hardy crop. *Physiology and Molecular Biology of Plants,* 24:551–561.

5 Lisar SYS, Motafakkerazad R, Hossain MM & Rahman IMM. 2012. Water stress in plants: Causes, effects and responses. In Ismile, Rahman, M. and Hasegawa, H. (Eds.), Water stress in plants (pp. 1-15). IntechOpen.

6 Farooq M, Wahid A, Kobayashi N, Fujita D & Basra SMA. 2009. Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development,* 29:185-212.

7 Duan B, Yang Y, Lu Y, Korpelainen H, Berninger F & Li C. 2007. Interactions between drought stress, ABA and genotypes in *Picea asperata*. *Journal of Experimental Botany,* 58:3025-3036.

8 Chaves MM, Flexas J & Pinheiro C. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany,* 103:551-560.

9 Ashraf M. 2009. Inducing drought tolerance in plants. *Biotechnology Advances,* 28:169-183.

10 Govind G, Gowda HVT, Kalaiarasi PJ, Iyer DR, Muthappa SK, Nese S & Makarla UK. 2009. Identification and functional validation of a unique set of drought-induced genes preferentially expressed in response to gradual water stress in peanut. *Molecular Genetics* and *Genomic,* 281:591-605.

11 Kumar A, Sharma S, Chunduri V, Kaur A, Kaur S, Malhotra M, Kumar A, Kapoor P, Kumari A, Kaur J, Sonah H & Garg M. 2020. Genome-wide identification and characterization of heat shock protein family reveal role in development and stress conditions in *Triticum aestivum* L. *Scientific Reports,* 10:7858.

12 Gurjar K, Rampuria S, Joshi U, Palit P, Bhatt K V, Mohapatra T & Sharma R. 2014. Identification of heat-related ESTs in mothbean through suppression subtraction hybridization. *Applied biochemistry and biotechnology,* 173:2116-2128.

13 Rampuria S, Joshi U, Palit P, Deokar AA, Meghwal RR, Mohapatra T, Srinivasan R, Bhatt KV & Sharma R. 2012. Construction and analysis of an SSH cDNA library of early heat-induced genes of *Vigna aconitifolia* variety RMO-40. *Genome* 55: 783-96.

14 Harsh A, Sharma YK, Joshi U, Rampuria S, Singh G, Kumar S & Sharma R. 2016. Effect of short-term heat stress on total sugars, proline and some antioxidant enzymes in moth bean (*Vigna aconitifolia*). *Annals of Agricultural Sciences,* 61:57-64.

15 Priya A M, Krishnan S R & Ramesh M. 2015. Ploidy stability of *Oryza sativa*. L cv. IR64 transformed with the moth bean *P5CS* gene with significant tolerance against drought and salinity. *Turkish Journal of Biology,* 39: 407-416.

16 Sharma R, Jain M, Kumar S & Kumar V. 2014. Evaluation of differences among *Vigna aconitifolia* varieties for acquired t tolerance. *Agricultural Research,* 3: 104-112.

17 Ortiz C & Cardemil L. 2001. Heat-shock responses in two leguminous plants: A comparative study. *Journal of Experimental Botany,* 52: 1711–1719.

18 Wang K, Zhang X, Goatley M & Ervin E. 2014. Heat shock proteins in relation to heat stress tolerance of creeping bentgrass at different N levels. *PLoS One,* 9: e102914.

19 Yong B, Wang X, Xu P, Zheng H, Fei X, Hong Z, Ma Q, Miao Y, Yuan X, Jiang Y & Shao H. 2017. Isolation and abiotic stress resistance analyses of a catalase gene from *Ipomoea batatas* (L.) Lam. *BioMed Research International,* 2017: 10.

20 Wang W, Cheng Y, Chen D, Liu D, Hu M, Dong J, Zhang X, Song L & Shen F. 2019. The *catalase* gene family in cotton: genome-wide characterization and bioinformatics analysis. *Cells,* 8(2): 86.

21 Koonin EV & Galperin MY. 2003. Principles and Methods of Sequence Analysis. In Boston (Ed.), Sequence - Evolution - Function: Computational approaches in comparative genomics, Kluwer Academic, 2003.

22 Koramutla MK, Ram C, Bhatt D, Annamalai M & Bhattacharya R. 2019. Genome-wide identification and expression analysis of sucrose synthase genes in allotetraploid *Brassica juncea*. *Gene,* 707: 126-135.

23 Polidoros AN, Mylona PV, Pasentsis K & Tsaftaris AS. 2003. Catalase expression in normal metabolism and under stress in the model legume *Medicago truncatula*. In Proceedings of International congress on Genes, gene families and isozymes, (pp. 147–156), Medimond Srl, Berlin.

24 Wu X, Li J, Tan J & Liu X. 2016. Molecular cloning, characterization and expression analysis of a catalase gene in *Paphia textile*. *Acta Oceanologica Sinica,* 35: 65–73.

25 Sharma S & Hooda V. 2018. Identification of coding sequence and its use for functional and structural characterization of catalase from *Phyllanthus emblica*. *Bioinformation,* 14: 8-14.

26 Klotz MG & Loewen PC. 2003. The molecular evolution of catalatic hydroperoxidases: evidence for multiple lateral transfer of genes between prokaryota and from bacteria into eukaryote. *Molecular Biology and Evolution,* 20:1098–1112.

27 Figueroa-Yanez L, Cano-Sosa J, Castano E, Arroyo-Herrera A L, Caamal-Velazquez J H, Sanchez-Teyer F, Lopez-Gomez R, Santos-Briones C D L & Rodrıguez-Zapata L. 2012. Phylogenetic relationships and expression in response to low temperature of a catalase gene in banana (*Musa acuminate* cv. "Grand Nain") fruit. *Plant Cell Tissue and Organ Culture*, 109:429-438.

28 Muthusamy SK, Sivalingam PN, Sridhar J, Singh D and Haldhar SM. 2017. Biotic stress inducible promoters in crop plants-a review. *Journal of Agriculture and Ecology* 4: 14-24.

Preferred citation: Meena MK, Dogra I, Sudarsan Y, Sharma AK, Singh D, Sharma V & Chet Ram. 2022. Identification and characterization of *CAT1* gene during drought stress in moth bean [*Vigna aconitifolia* (Jacq.) Marechal]. *Journal of Agriculture and Ecology*, 14: 164-172; http://doi.org/10.53911/JAE.2022.14224