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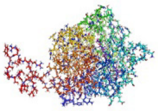

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3D model of OsTHIC

Prediction and Validation of 3-Dimensional Structure of Rice *OsTHIC* Abiotic Stress Responsive Gene

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ABSTRACT

Rice is an important cereal crop believed to have been cultivated for hundred years. The ancestry and evolution of this plant and its diverse cultivars remain contentious. Thus, determining the cultivar's molecular mechanism of abiotic stress-responsive genes using a bioinformatics system continues to be an area to investigate. For better knowledge of the mechanisms of the abiotic stress-responsive gene from rice, a novel stress-related gene named OsTHIC was selected for this study. The OsTHIC gene accessions were collected from NCBI, then predict its secondary structure. I-TASSER server was used to predict the 3-dimensional [3D] model of the gene protein and validated using ERRAT and Ramachandran plot. The OsTHIC gene appeared to be highly conserved and structurally functional. The secondary structure of the OsTHIC indicated a high percentage of a random coil [47.35%], followed by an alpha helix [45.05%], then an extended strand [10.61%]. The prediction using I-TASSER modeller produced structures based on the protein sequence. The protein 3D model showed different model qualities using the two-refinement software which ERRAT Plot model refinement having the best score at 97.266%.

INTRODUCTION

Rice [*Oryza sativa*]; is a primary crop, and carbohydrate provider for more than two-quarter of the world's populace [1, 2]. *Oryza sativa* production is mostly in sub-tropical and tropical environs as ascendant cereal. The significance of this crop plant as a diet can never be emphasized and differs among cultivating zones and countries [3]. Interestingly, the crop has close relation with other cereals, considered a model and prototypical taxonomic group for gene and protein analysis through the use of molecular knowledge including gene expression through stress-resistant and bioinformatics analysis [4]. Abiotic stresses are the antagonistic impact of non-living factors on the living organisms in a particular ecosystem or environment and continue to be one of the most critical challenges facing crop production worldwide. Abiotic stresses have an intense influence on rice productivity, growth and development [5]. To live under abiotic stresses, flora species have to evolve strategies for responding to various abiotic stresses as they cannot physically transfer from environmental stresses [6]. Adaptation to the said condition and ability to respond ultimately may be an impulsive force in speciation as discussed by Lexer and Fay [7]. It has been demonstrated that because of the complex nature of abiotic stress, many signalling pathways are activating during exposure to either of the stresses which are likely to be amenable to the perception of the tension. Considering that, stress-induced gene merchandise was divided into stress signal transduction and stress tolerance. A Similar was reported by Rabbani, Maruyama [8] who demonstrated the overlap between abiotic stresses during gene expression. In recent years, cellular and molecular mechanisms have attracted more attention in providing more details information on any biological activity [9, 10].

OsTHIC genes were derived from non-biological stress in non-biological constraints and serve an important role in the reaction to various constraints on rice [11]. The gene present in rice makes it plays a crucial role in response to various abiotic stresses such as heat, cold and drought. To improve the resistance to the biological stress of rice, plants must develop mechanisms sensitive to those stress-providing factors such as heat, oxidative stress, osmotic pressure, salt, cold and fertilizer constraints [12]. Exposure of the rice varieties to utmost environmental circumstances led the plant to a dramatic diminution in agronomic traits. However, to improve abiotic stress resistance in rice it is paramount to understand the mechanisms by which plants respond to these abiotic stresses. Recently, the molecular mechanisms underlying the adaptation of plant species to environmental stresses have attracted the attention of many researchers, hence, determining the molecular structure of the gene[s] responsible remains a golden ticket in computational biology. Determining the structure of the OsTHIC gene will provide insight into the molecular mechanisms of the gene activation and repression and serve as a reference for future wet lab analysis**.**

MATERIALS AND METHODS

Collection of OsTHIC Gene Accessions

OsTHIC gene protein sequence was retrieved from the National Centre for Biotechnology Information [NCBI] [http://www.ncbi.nlm.nih.gov/]. The OsTHIC protein full-length sequences from the GeneBank were analyzed using BLASTp algorithm. Then, later translated the protein sequence to nucleotides using ExPASy [http://web.expasy.org/translate/] as earlier described by Mohammed, Ndejiko [9].

Prediction of OsTHIC Gene Secondary Structure

The OsTHIC gene primary protein sequence was subjected to secondary structure prediction analysis. The secondary structure was predicted using NPS-GOR4 tool by the information theory algorithm [13, 14].

3-Dimensional (3D) Structure Modeling of the OsTHIC Gene

The OsTHIC protein 3D structure was generated using I-TASSER (Iterative-Threading / Assembly/Refinement). The modeller always gave a prediction of the structures based on protein primary sequence. Whereas, the Quaternary structure was predicted and modelled using PYMOL software.

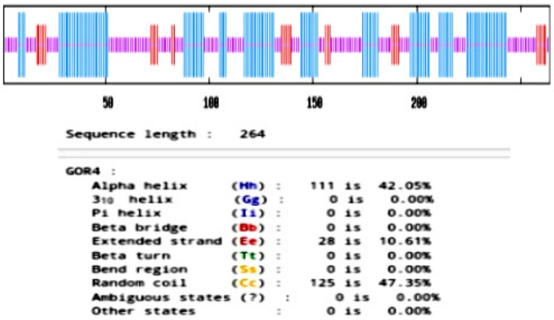
Validation Analysis of the 3D Models of OsTHIC Gene

Respective OsTHIC 3D models generated were assessed for stereochemistry quality. The validation of the predicted 3D models was made for the correctness of the overall fold and structure using the Ramachandran plot and ERRAT plot from RAMPAGE server bioinformatics tools [15].

RESULTS AND DISCUSSION

OsTHIC Gene and Its Secondary (2D) Structure

In this bioinformatics analysis, the OsTHIC gene primary sequences deposited in the NCBI genebank were successfully retrieved and analyzed for nucleotide sequence. The OsTHIC protein secondary structure prediction was achieved using NPS@:GOR4 software (**Fig. 1**). Secondary structures of protein including α-helix, β-sheet, and loops were observed on the OsTHIC protein structure. The secondary structure composition analysis results showed that α-helix is 45.05%, the extended strand is only 10.61% and the maximum random coil of 47.35%. The protein structures were comprehensively scrutinized for quality selection and relationship [16, 17].



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Sequence length :   264

GOR4 :
   Alpha helix       (Hh) :   111 is  42.05%
   3₁₀ helix         (Gg) :     0 is   0.00%
   Pi helix          (Ii) :     0 is   0.00%
   Beta bridge       (Bb) :     0 is   0.00%
   Extended strand   (Ee) :    28 is  10.61%
   Beta turn         (Tt) :     0 is   0.00%
   Bend region       (Ss) :     0 is   0.00%
   Random coil       (Cc) :   125 is  47.35%
   Ambiguous states  (?)  :     0 is   0.00%
   Other states           :     0 is   0.00%
```

Fig. 1. the results of OsTHIC showing secondary structure prediction using NPS@:GOR4.

OsTHIC Gene 3D Structure Prediction

The OsTHIC gene 3D structure was generated using I-TASSER. The modeller software produced a prediction of the model based on its protein primary sequence. Subsequently, the 3D models' quality was validated and assessed for stereochemistry in the RAMPAGE server using the Ramachandran plot and ERRAT plot. The molecular model generated by the server has good quality and resolution based on C-score. Further, the measurement based on C-score quality resulted in good models and demonstrated their correlation quality [18]. **Fig. 2** showed the OsTHIC gene 3D model generated.

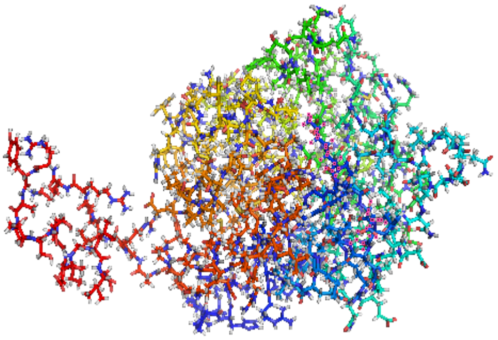

Fig. 2. The 3D model structure of OsTHIC gene.

Moreover, the I-TASSER server was discovered to provide more quality models and good viewing, albeit, a complex formulation used in the last two CASP experiments. The server is an integrated program for automated protein 3-dimensional modelling, prediction and determinant of their functions based on the sequence-to-structure to function paradigm [9,18]. The 3D model is used to determine the biological function of protein building blocks, as well as describe the interaction of protein-ligand or protein-protein. Therefore, the most frequent motivation for protein 3D model prediction is to subject toward getting insight into the proteins' molecular activities and interaction with other proteins or ligands. The OsTHIC 3D prediction analysis could help in exploring and determining more the molecular function[s] of this novel abiotic stress protein and encouraging further investigation of other rice abiotic stress resistant genes, interaction and function.

Validation of Refinement Model

3D models of the representative accessions were selected for the validation analysis using Ramachandran plot and ERRAT plot tools. **Fig. 3** showed the result of the Ramachandran plot with a total quality score of 89.778%. White black-grid represents preferred conformations -2 > Delta >= -4 and white with grey-grid represents questionable conformations Delta < -4. Whereas, highly preferred observations were shown as GREEN crosses: 202 [89.778%] and preferred observations were shown as BROWN Triangles: 20 [8.889%], then questionable observations were shown as RED Circles: 3 [1.333%].

The Ramachandran model validation tool ascertains that the obtained structure is presentable preceding to the beginning of any more robust analysis. Early reports indicate that 80-90% favoured regions [core region] in the protein measured as a high-quality model (**Fig. 3**) [19].

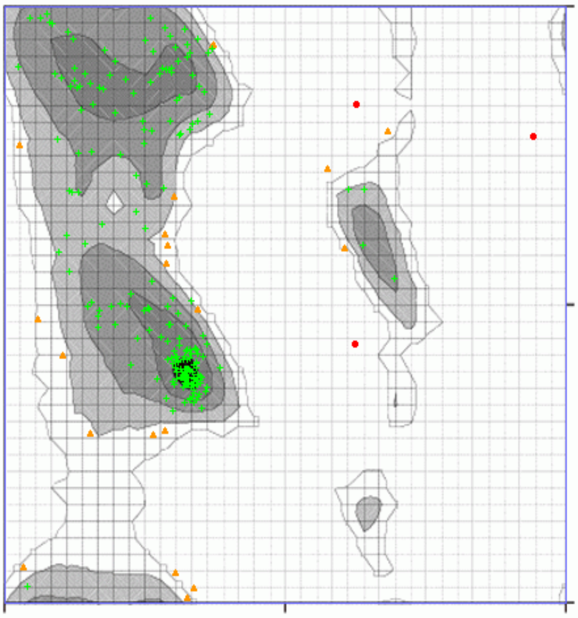

Fig. 3. The OsTHIC gene 3D structure validation and refinement using Ramachandran plot.

The model refinement quality verified using ERRAT plot software gave the best score at 97.226% (**Fig. 4**). With this optimum score from ERRAT established, it is obvious that the quality model was generated from the present study. Reports indicated that the presence of 80–90% of residues “favoured region” [core region], as well as, the leftover residues in the “additional allowed” region bespeak that the model generated has a well-refined construction and structures. Furthermore, any model with more than 90% favoured regions is considered a high-quality model. ERRAT plots normally measure the C-score, determine the quality of resulting models and demonstrate their correlation quality [18]. The ERRAT plot analyzes the statistics of non-bounded interactions between diverse atom types. Further, plot a graph of a value of error function versus position of a 9-residue sliding window, calculated by the comparison with statistics from highly refined structures. From the results displayed by ERRAT Plot, the best OsTHIC 3D structure was predicted.

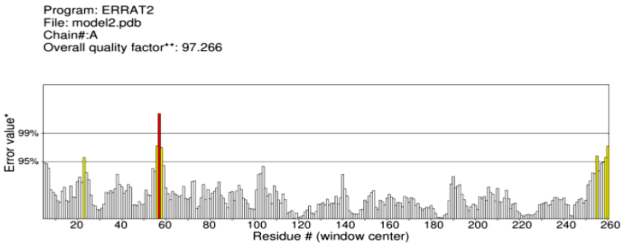

Fig. 4. The OsTHIC gene 3D structure validation and refinement using ERRAT plot.

CONCLUSION

OsTHIC is an abiotic stress-responsive, newly discovered gene found in rice. The gene was found to be resistant to abiotic stresses including temperature [hot or cold] and drought. The OsTHIC gene structural modelling carried out in this study revealed the secondary structure, as well, as the 3D model which was constructed using the I-TASSER server. The 3D model validation based on the C-score indicated a high-quality model. The ERRAT plot results displayed the best validation of OsTHIC 3D structure at 97.226% model quality. This will help in knowing the molecular function of this valuable abiotic stress-sensitive gene, its suppression and activation mechanism networks and inspire advanced research. Hence, wet laboratory analyses of the OsTHIC gene to further understand its additional function and molecular mechanism in rice need to carry out.

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