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Homology Modeling and Microarray Analysis of Silicon Transporter Protein in Rice, Barley and Maize

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Abstract: Higher plants like rice, barley and maize have unique silicon accumulation characteristic. Silicon is taken up in the form of silicic acid by silicon transporter protein in root. The uniqueness of silicon accumulation in different plants is caused by both the physiologic and molecular diversity in the species even in different parts of a single plant. To understand the mechanism of silicon or silicic acid uptake, it is essential to analyze and study the 3D structure of transporter protein and expression profile through microarray experiments. We used I-TASSER and RAMPAGE for prediction and validation of 3D structure of silicon transporters respectively followed by microarray analysis. The 3D models those showed over 90% residues in favorable regions were considered in this study. Microarray analysis indicated root and shoot with highest silicon accumulation in the analyzed plants. For the development of better abiotic stress tolerant plant, expression of this transporter protein in shoot and root is given priority. This particular work might be helpful for designing a better plant with target efficient and maximum silicon uptake ability.

Keywords: Silicon Transporter; Biotic Stress; Abiotic Stress; 3D Structure; Microarray

1. INTRODUCTION

Silicon (Si) is an advantageous element for the growth of plant [1]. It aids plants to overcome various biotic and abiotic stress [2, 3]. Si helps to fight against both fungi and bacterial diseases caused by increasing resistance of various plant species [4]. Si enhances resistance against diseases like rice blast, powdery mildew, and sheath blight[5]. It also reduces insect pests[6]. It helps in suppressing stem borer and brown plant hopper [7]. Interestingly, silicon improves nutrient imbalance in a wide variety of plant

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Table 1: Silicon Transporter Protein for Rice, Barley and Maize

Accession number	Source	Amino acid residue
90855460	*Oryza sativa* Japonica group	298
224548822	*Hordeum vulgare*	295
99866966	*Zea mays*	301

Table 2: InterProScan-Simplified presentation

Accession number	Source	Aquaporin or aquaporin like domain
90855460	*Oryza sativa* Japonica group	YES
224548822	*Hordeum vulgare*	YES
99866966	*Zea mays*	YES

Table 3: Dataset for multiple sequence alignment

Accession number	Source	Amino acid residue
75294117	*Oryza Sativa* Japonica group	298
90855460	*Oryza Sativa* Japonica group	298
193811876	*Oryza Sativa* Japonica group	298
99866966	*Zea mays*	301
145228075	*Sorghum bicolor*	297
148467568	*Sorghum bicolor*	295
304651330	*Triticum aestivum*	295

Table 4: List of gene ID retrieved from PLEXdb

Accession number	Source	Length	Gene location ID
75294117	*Oryza sativa* Japonica group	298	LOC_Os06g12310.1
90855460	*Oryza sativa* Japonica group	298	LOC_Os06g12310.1
152717100	*Oryza sativa* Japonica group	472	LOC_Os03g01700.1
193811876	*Oryza sativa* Japonica group	298	LOC_Os06g12310.1
296936086	*Oryza sativa* Japonica group	472	LOC_Os03g01700.1
253960506	*Hordeum vulgare*	474	LOC_Os02g57620.1
224548822	*Hordeum vulgare*	295	LOC_Os06g12310.1
253960504	*Hordeum vulgare*	300	LOC_Os06g12310.1
308044363	*Zea mays*	477	LOC_Os03g05390.9
256997236	*Zea mays*	477	LOC_Os03g05390.9
99866966	*Zea mays*	301	LOC_Os02g51110.1

species [8]. In addition, Silicon enhances the resistance to lodging, alleviates metal toxicity, salt and drought stresses [4].

Every plant contains silicon in their tissue[9] but differs in accumulation ranging from 0.1% to 10.0% of shoot dry weight[10]. Silicon is taken up by the root in the form of silicic acid, an uncharged molecule[11, 12]. Three Si transporters have been acknowledged to be involved in both the uptake and distribution of silicon denoted as Lsi1, Lsi2, and Lsi6. These are identified from rice which is considered

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to be a characteristic Si-accumulating species [10, 13, 14].Water-selective channel proteins which are too recognized as aquaporins (AQPs) involved with transmembrane water flow. During growth and development processes like germination, cell elongation, stomatal movement, phloem loading and unloading, AQPs play role to mediate and regulate rapid transmembrane water flow[15, 16]. In addition, reproductive growth and stress responses are also mediated and controlled by AQPs[16, 17].

Based on amino acid sequence homology and protein subcellular localization, plant AQPs family is classified into four families[18, 19]. Namely, plasma membrane intrinsic proteins (PIPs)[20]; tonoplast membrane intrinsic proteins (TIPs)[21]; nodulin 26-like intrinsic proteins (NIPs)[22]; and small basic intrinsic protein (SIPs)[23]. Lsi1 and Lsi6 belong to the nodulin-26 like major intrinsic protein III (NIP III) subgroup[10, 24]. Identifying salt tolerance genes and understanding their functions have become the most urgent tasks in agricultural research today. Scientists around the globe are trying to make new varieties which are tolerant to biotic and abiotic stress. Rice has silicon transporter and higher the uptake of silicon, better the tolerance against biotic and abiotic stress.

Here our aim was to construct 3D model of silicon transporter protein in higher plants. For this we have selected the transporter proteins available in parts (root, shoot) of rice, barley and maize. In the next step we identified the presence of aquaporin or aquaporin-like domain in rice, barley and maize related to stress tolerance. After identification of necessary domain and motif, we proceeded for construction of 3D models. In this step, sequentially we used number of tools like SOSUI for transmembrane domain prediction, I-TASSER for 3D structure prediction. C-score, TM-score guided models were validated by RAMPAGE.

2. METHODS AND MATERIALS

2.1 Data Set Collection

The sequence of silicon transporter in Rice (75294117) was retrieved from National Center for Biotechnology Information (NCBI). Basic Local Alignment Search Tool (BLAST) of the retrieved sequences was done through NCBI in order to find the sequence homology with the sequence of silicon transporter in Rice (75294117). The retrieved sequences through BLAST for the same transporter from Rice and other higher plant species are enlisted as follows in Table1.

2.2 Domain Prediction

Domain prediction was done by InterProScan (V4.8;http://www.ebi.ac.uk/Tools/pfa/iprscan/).The collected sequences (*Oryza sativa* Japonica group; accession number: 90855460, *Hordeum vulgare*; accession number: 224548822 and *Zea mays*; accession number: 99866966) were run through InterProScan to filter the dataset. A simplified presentation of InterProScan output is enlisted in Table 2.

2.3 Multiple Sequence Alignment

Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) was used for Multiple Sequence Alignment (MSA) of the protein sequences. Both homology and the evolutionary relationships between the sequences studied can be deduced from the output. Multiple sequence alignment done for proteins is enlisted in Table 3.

2.4 Transmembrane Domain Prediction

Transmembrane domain prediction was done for the silicon transporter proteins through SOSUI (http://bp.nuap.nagoya-u.ac.jp/sosui/sosuiG/sosuigsubmit.html). Here sequences (*Oryza sativa* Japonica group; accession number: 90855460, *Hordeum vulgare*; accession number: 224548822 and *Zea mays*; accession number: 99866966) were used for prediction.

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2.5 3D Structure

3D structure of the transporter proteins were generated through I-TASSER (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) server. For 3D structure generation, sequences of Rice (*Oryza sativa* Japonica group; accession number: 9085546), Barley (*Hordeum vulgare*; accession number: 224548822) and Maize (*Zea mays*; accession number: 99866966) were used as input. Starting from an amino acid sequence, we generated three-dimensional (3D) atomic models from multiple threading alignments and iterative structural assembly simulations using I-TASSER. The function of the protein was then inferred by structurally matching the 3D models with other known proteins. The output from a typical server run contain full-length secondary and tertiary structure predictions, and functional annotations on ligand-binding sites, Enzyme Commission numbers and Gene Ontology terms.

Accuracy of the predicted models by I-TASSER was provided based on the confidence score (C-score) of the modeling. It was calculated based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. C-score is typically in the range of -5 to 2, where a C-score of higher value signifies a model with a high confidence and vice-versa.

TM-score and RMSD are known standards for measuring structural similarity between two structures which are usually used to measure the accuracy of structure modeling when the native structure is known. In case where the native structure is not known, it becomes necessary to predict the quality of the modeling prediction, i.e. what is the distance between the predicted model and the native structures. For that we tried the TM-score and RMSD of the predicted models relative the native structures based on the C-score.

TM-score is a recently proposed scale for measuring the structural similarity between two structures. The purpose of proposing TM-score is to solve the problem of RMSD which is sensitive to the local error. Because RMSD is an average distance of all residue pairs in two structures, a local error will raise a big RMSD value although the global topology is correct. In TM-score, however, the small distance is weighted stronger than the big distance which makes the score insensitive to the local modeling error. A TM-score >0.5 indicates a model of correct topology and a TM-score<0.17 means a random similarity. These cutoffs do not depend on the protein length.

I-TASSER generates full length model of proteins by excising continuous fragments from threading alignments and then reassembling them using replica-exchanged Monte Carlo simulations. Low temperature replicas (decoys) generated during the simulation are clustered by SPICKER and top five cluster centroids are selected for generating full atomic models. The cluster density is defined as the number of structure decoys at a unit of space in the SPICKER cluster. A higher cluster density means the structure occurs more often in the simulation trajectory and therefore signifies a better quality model.

2.6 3D Structure Validation

RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) was applied for structural validation by the Ramachandran plot assessment. Based on a manually selected set of high-quality protein structures (from the Richardson's Group at Duke University) and a number of filters (such as B-factor cutoff and van der Waals clashes), reference phi/psi plots were derived for Gly, Pro, pre-Pro and general (other) residue types, and subdivided into "favored", "allowed" and "outlier" regions.

2.7 PLEXdb (Plant Expression Database)

The gene location ID for the desired protein was retrieved from PLEXdb (http://www.plexdb.org). BLAST tool was chosen from PLEXdb and protein-protein BLAST was

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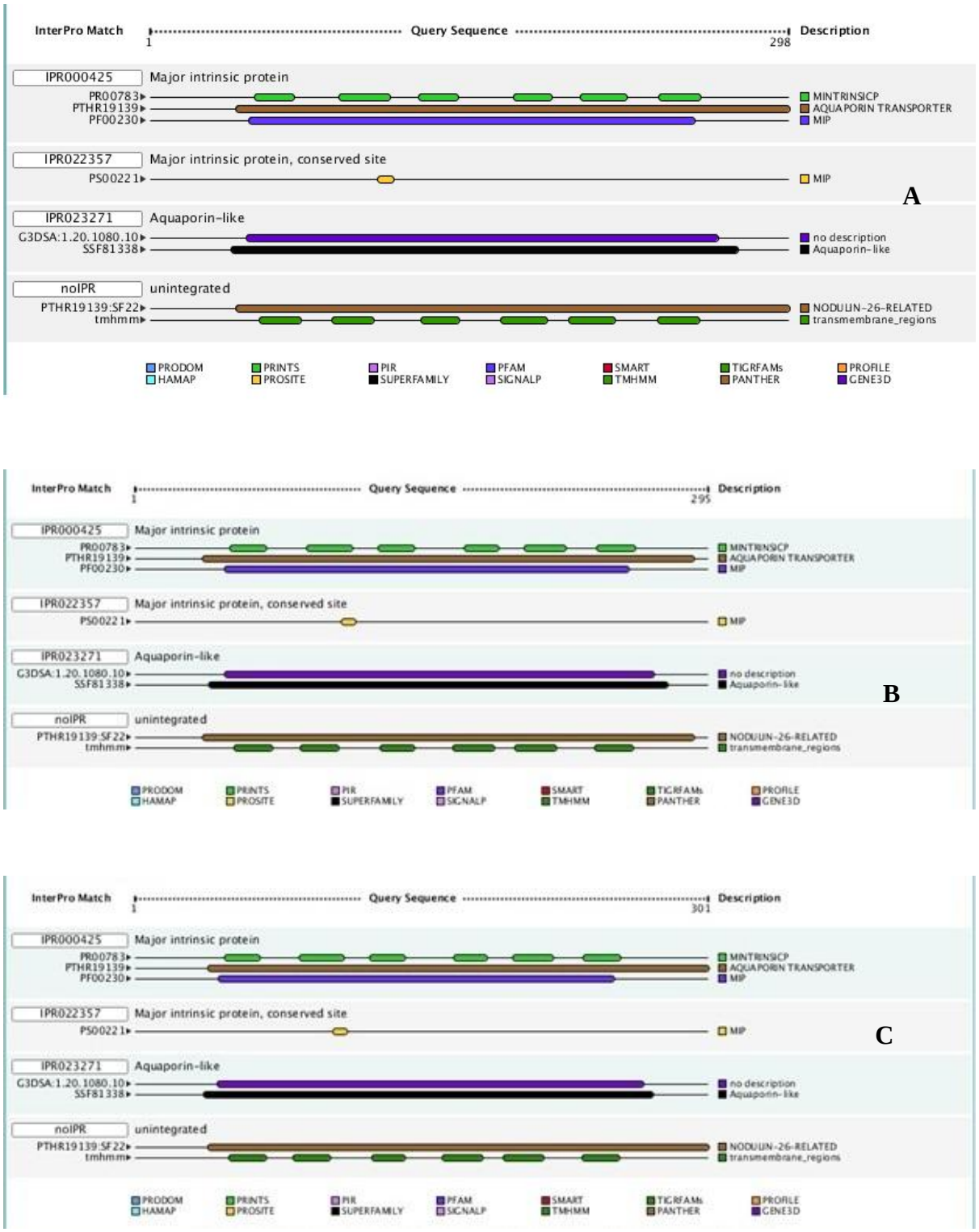

Figure1: A) InterProScan output for *Oryza sativa* Japonica group (Accession number: 90855460) B) InterProScan output for *Hordeum vulgare*(Accession number: 224548822) C) InterProScan output for *Zea mays* (Accession number: 99866966)

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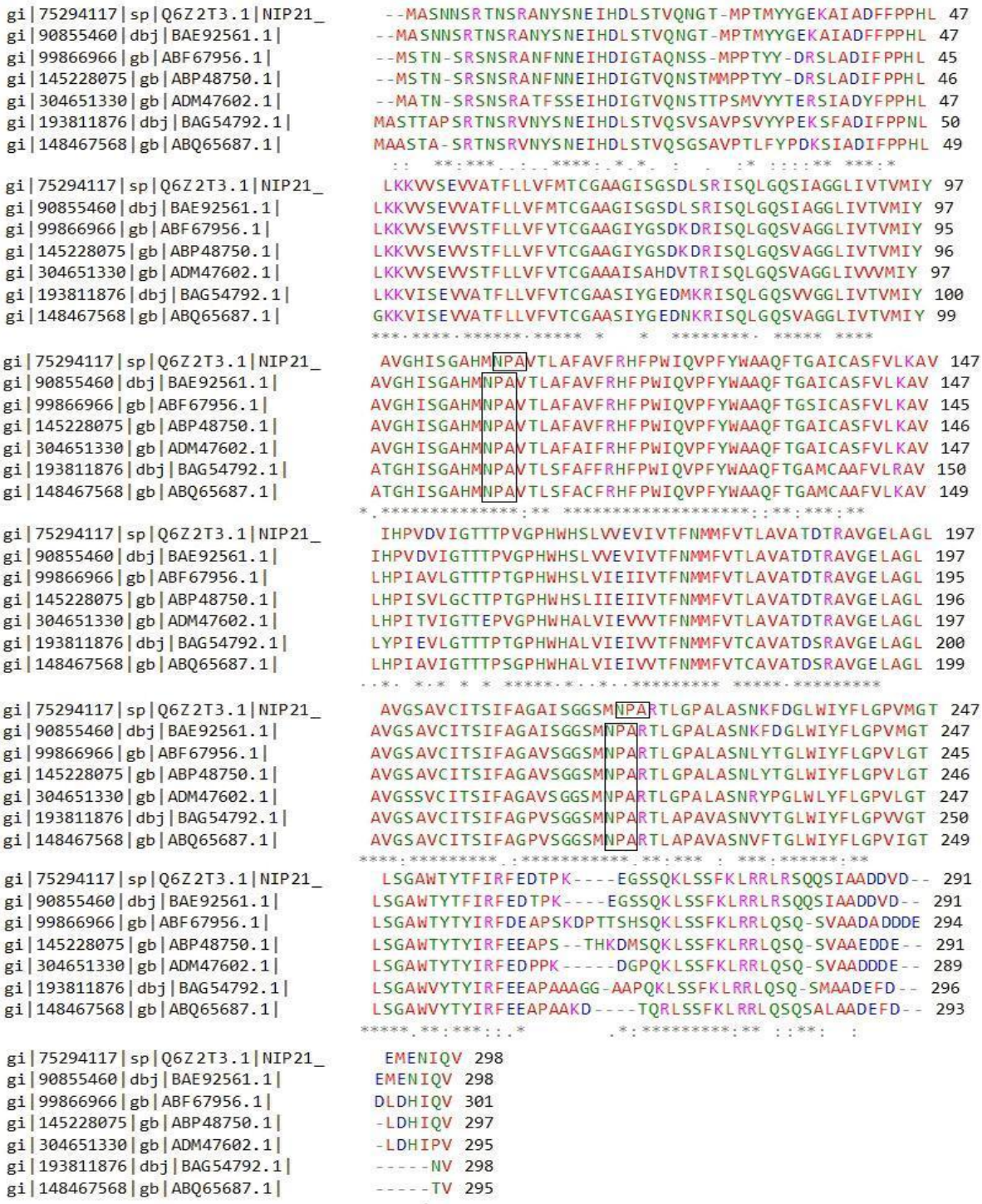

Figure 2. Multiple sequence alignment of silicon transporter proteins in *Oryza sativa* and other higher plants like *Hordeum vulgare*, *Zea mays*, *Sorghum bicolor*, *Triticum aestivum*

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performed. FASTA sequence data was placed as input which is the supported format to BLAST against the consensus sequence for all probe sets on the selected target array of Arabidopsis ATH1 22k. The input consists of probe set IDs are blasted against the consensus sequence for the selected target array. From the BLAST result, we picked the best identical match to find the gene location ID. The NCBI accession numbers, plant sources, length and gene location ID retrieved from PLEXdb are enlisted in Table 4. PLEXdb BLAST was performed to search the PLEXdb database for matching exemplars from microarray platforms which BLAST sequence against the consensus sequence for each microarray.

2.8 Rice Oligonucleotide Array Database (ROAD)

Gene list was imported directly for microarray experiments of ROAD (http://www.ricearray.org/index.shtml). Genome wide expression profiling of rice (GSE7951) was selected to identify the expression level in root and shoot [25].

3. RESULTS

3.1 Domain Prediction

InterProScan provides functional analysis of proteins by classifying them into families and predicting domains and important sites. The result for *Oryza sativa* Japonica Group (Accession number 90855460), *Hordeum vulgare* (Accession number: 224548822) and *Zea mays* (Accession number: 99866966) was shown in Figure 1 (A, B and C) where aquaporin and aquaporin-like domain is present in all silicon transporters of the plants.

3.2 Multiple Sequence Alignment

Multiple sequence alignment was done to identify Asn-Pro-Ala (NPA) motif in the same silicon transporter protein sequences. The alignment result and confirmation of the presence of NPA motif are shown in Figure 2.

3.3 Transmembrane Domain Prediction

Transmembrane domain prediction was done here to prove them as transporter proteins. Transmembrane domain needs at least 18 residues to span the membrane. The entire predicted domain has length approximately 23. And the regions of transmembrane domain in all sequences are almost same. The prediction result for *Oryza sativa, Hordeum vulgare and Zea mays* is summarized in Table S1-S3 respectively.

3.4 3D Structure

The three dimensional modeling of these transporter sequences is the crucial task as there is no PDB files were found for these sequences, except one. Here we used I-TASSER for homology modeling. All the results and the image of best predicted model were enlisted. Figure 3(A, B & C) showed the model for *Oryza sativa, Hordeum vulgare and Zea mays* respectively.

C-score is used to estimate the accuracy of predicted model by I-TASSER. The typical C-score value ranges between -5 to 2, where a higher C-score value signifies a model with a high confidence and vice-versa. In table 5(b), 6(b) and 7(b), we observe that C-score for *Oryza sativa, Hordeum vulgare and Zea mays* is -0.10, 0.11 and 0.14, respectively. These values are the indicator of good model. The value of other parameters (No. of decoys & cluster density) generated by I-TASSER for the significance of model

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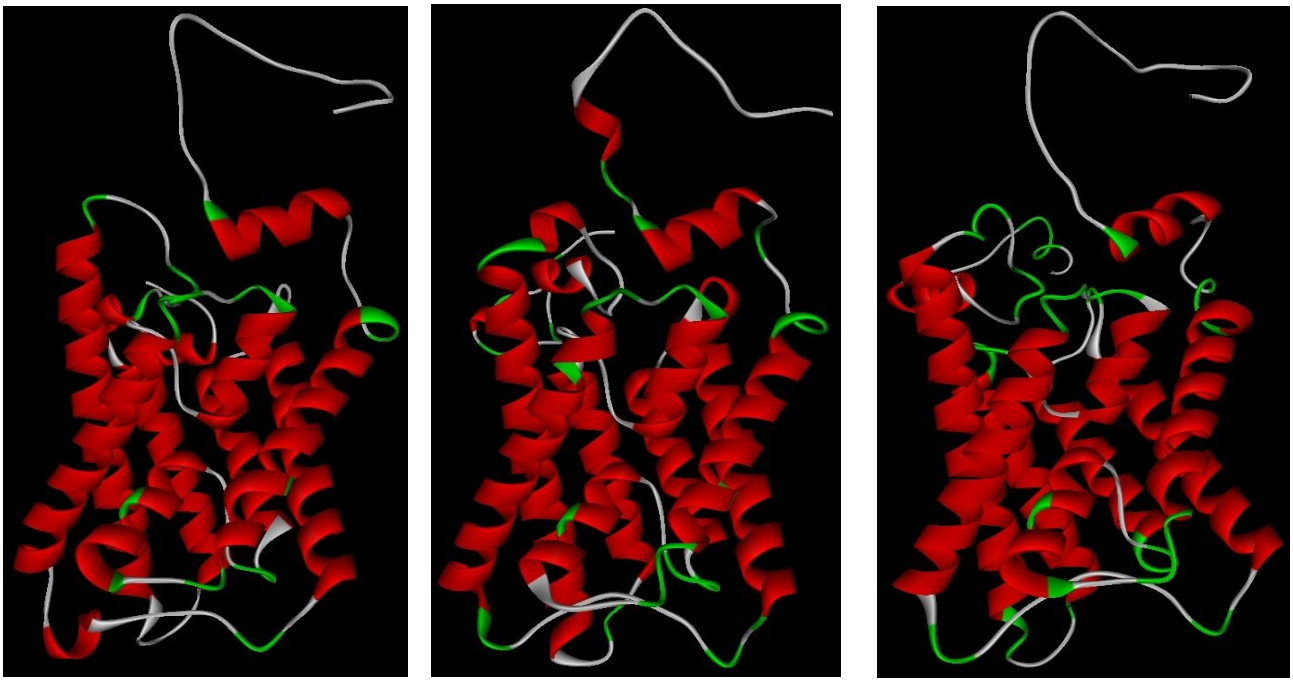

(A) **(B)** **(C)**

Figure 3: (A) 3D structure for *Oryza sativa* Japonica group (Accession number: 90855460) (B) 3D structure for *Hordeum vulgare* (Accession number: 224548822) (C) 3D structure for *Zea mays* (Accession number: 99866966)

shown in table 5(b), 6(b) and 7(b) are in reliable range.

TM-score for rice, barley and maize is 0.70, 0.73 and 0.73 respectively. Table 5(a), 6(a) and 7(a) represents TM-score and RMSD value for rice, barley and maize, respectively.

3.5 RAMPAGE

Structural validation of 3D models of silicon transporter was done by RAMPAGE. The number of residues in allowed or disallowed regions of the Ramachandran plot (Figure 4) determines the quality of the model. Number of residues in favored region for rice, barley and maize were 93.6%, 93.2% and 82.6%, respectively.

3.6 ROAD

The result found in microarray experiment showed a good expression status- based on expression scale incorporated with the heat map, in both shoot and root compared with other parts of the plant for almost every gene location ID retrieved from PLEXdb. The output for microarray was shown in Figure 5.

4. DISCUSSION

The recent studies describe that the more Si accumulated in the shoots, the larger the beneficial effects of Si under stress conditions. Although Si is abundant in soil, since most plants are unable to take up a large amount of Si from soil, they do not benefit from Si. In our study, we also found the expression in shoot and root. As it is known that rice is a typical Si-accumulating plant so we analyzed the sequence

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Table 5 (a): I-TASSER output for *Oryza sativa* Japonica Group (Accession number: 90855460)

Accession number	Type	TM-score	RMSD
90855460	*Oryza sativa* Japonica Group	0.70±0.12	6.4±3.9Å

Table 5 (b): Score, No. of decoys, Cluster density value for different models (Accession number: 90855460)

	Model 1	Model 2	Model 3	Model 4	Model 5
Score	-0.10	-1.33	-0.47	-3.19	-1.76
No. of decoys	6881	2007	4756	312	1303
Cluster density	0.2108	0.0615	0.1457	0.0096	0.0399

Table 6 (a): I-TASSER output for *Hordeum vulgare* (Accession number: 224548822)

Accession number	Type	TM-score	RMSD
224548822	*Hordeum vulgare*	0.73±0.11	5.9±3.7Å

Table 6 (b): Score, No. of decoys, Cluster density value for different models (Accession number: 224548822)

	Model 1	Model 2	Model 3	Model 4	Model 5
Score	0.11	-0.99	-2.44	-1.27	-0.64
No. of decoys	6937	2316	541	1748	3633
Cluster density	0.2576	0.086	0.0210	0.0649	0.1349

Table 7 (a): I-TASSER output for *Zea mays* (Accession number: 99866966)

Accession number	Type	TM-score	RMSD
99866966	*Zea mays*	0.73±0.11	5.9±3.7Å

Table 7 (b): Score, No. of decoys, Cluster density value for different models (Accession number: 99866966)

	Model 1	Model 2	Model 3	Model 4	Model 5
Score	0.14	-0.6	-0.29	-0.95	-1.13
No. of decoys	6732	3200	4387	2264	1888
Cluster density	0.2665	0.1267	0.1737	0.0896	0.0747

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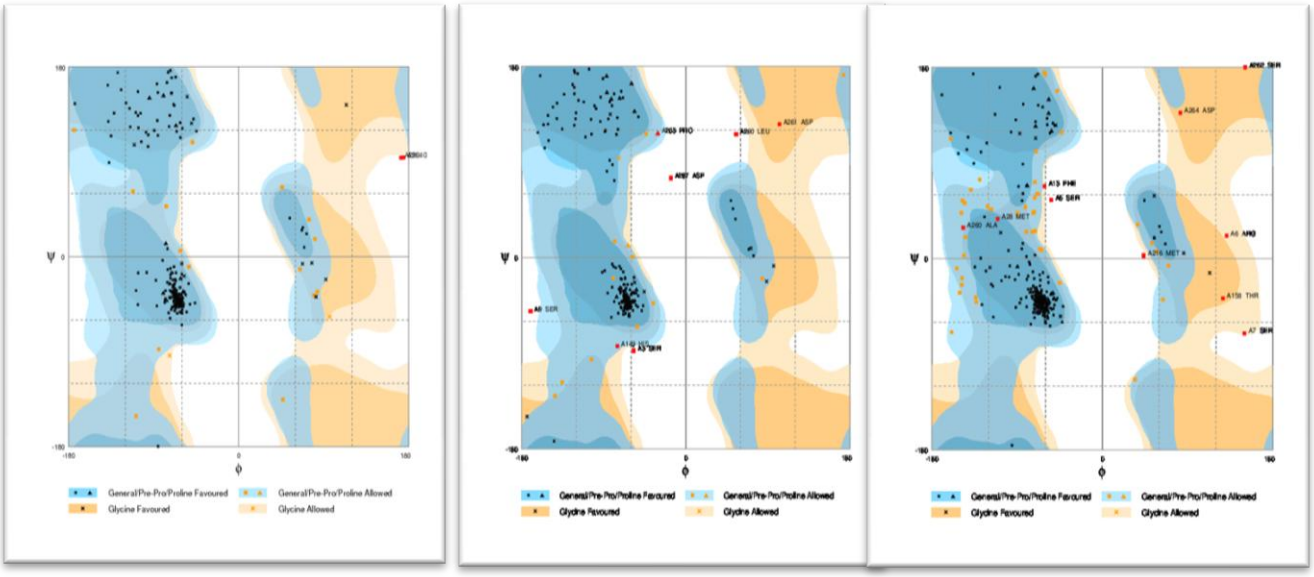

(A) **(B)** **(C)**

Figure 4: (A) Ramachandran plot for *Oryza sativa* Japonica group (Accession number: 90855460) (B) Ramachandran plot for *Hordeum vulgare* (Accession number: 224548822) (C) Ramachandran plot for *Zea mays* (Accession number: 99866966)

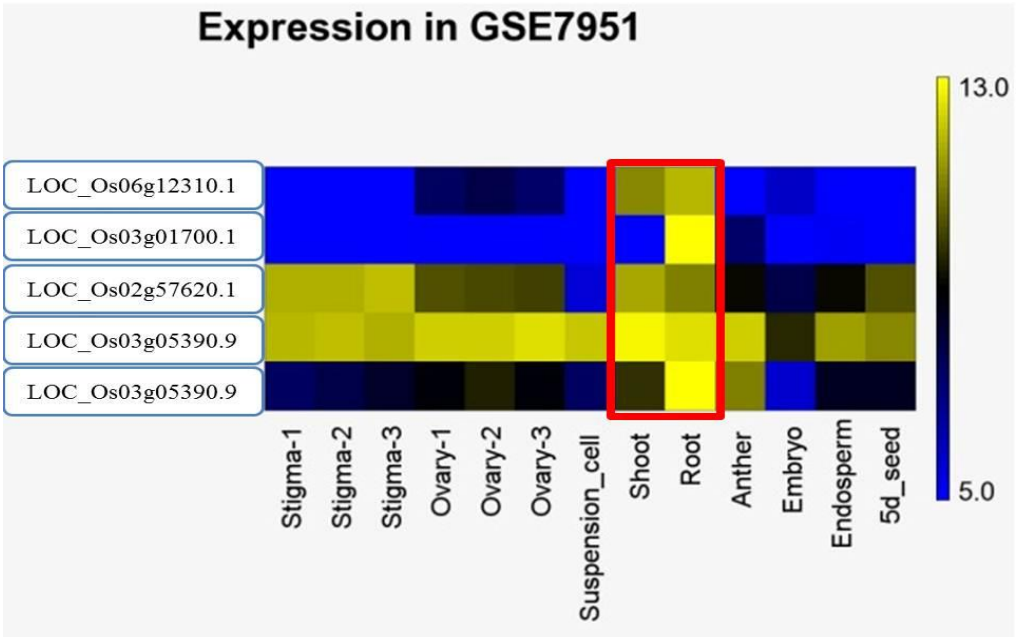

Figure 5: Microarray expression profile analyzed by Rice Oligo Array Database

and found the similar characteristic protein in barley and maize. The well known approach to enhance the resistance of plants to multiple stresses is genetic modification for Si uptake ability. To understand the different uptake ability of rice compared to other plants we generated 3D structure of protein. Protein

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sequences for the study were collected from NCBI as the starting step. These sequences were then run through InterProScan to identify aquaporin or aquaporin-like domain. For rice, barley and maize plants, there were the expected domains. Lsi1gene controls silicon accumulation in rice and is specific for silicon transport. This Lsi1 gene belongs to aquaporin family and it has already been known that the aquaporin is related to stress tolerance. The identification of a silicon transporter provides an insight into the silicon uptake system in plants. After successful identification of essential domain, presence of NPA motif was checked next step by Clustal Omega (multiple sequence alignment tools). The result from multiple alignments confirmed the presence of NPA motif, responsible for better stress tolerance. In the subsequent step, SOSUI was used for the transmembrane domain prediction. SOSUI results have revealed that all the sequences of this study have six transmembrane helix and all the helixes are started and ended almost at the same position. After primary analysis of sequences we proceeded to predict 3D structure by I-TASSER. Estimated C-score by I-TASSER was reliable for rice (-0.10), barley (0.11) and maize (0.14). Another important parameter TM-score for rice (0.70), barley (0.73) and maize (0.73) has also verified the structural accuracy. The generated models were validated by RAMPAGE also. All the above results imply that the sequence analysis and generated models are acceptable and keep scope for further study. Finally, the result found in microarray experiment shows a good expression status in both shoot and root compared to other parts of the plant for almost every gene location ID. Further study with structural information will provide valuable information for understanding difference and designing plants with better uptake ability.

5. CONCLUSION

In this study our goal was to predict the 3D structure for silicon transporter protein in rice, barley and maize. For that purpose presence of NPA motif was confirmed first. The result indicates the presence of NPA motif twice for the studied sequences almost at the same regions resembling aquaporin in conservancy. Thereafter, studied sequences confirm six transmembrane helixes and to be almost at the same position indicating structural similarity. After confirmation of relevant motif and structural similarity, I-TASSER was used for 3D structure prediction and the result was satisfactory to get reliable model. All the predicted models were validated by RAMPAGE and study shows the reliability as well as acceptance for the predicted models of rice, barley and maize. Eventually, expression profile through microarray result in shoot and root shows the prospect of silicon transporter. The findings of this study will be useful for making new varieties which are tolerant to biotic and abiotic stress.

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The authors declare no conflict of interest