Research Article

Isolation and In Silico Analysis of *Os*GLP12-3 Gene's Promoter from Rice Cultivar Dilrosh-97

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Abstract | Germins and Germin-like proteins (GLPs) are the ubiquitous family of plant proteins that belong to the cupin superfamily and have been reported to play a major role in plant defense against pathogens attacks and different abiotic stresses. Current research deals with the study of cis-regulatory elements located in the promoter region of *Os*GLP12-*3* gene of Nipponbare and newly sequenced *Oryza sativa L.* cultivar Dilrosh-97 using bioinformatics approach. The possible cis-acting regulatory elements were analyzed using PlantCARE and PlantPAN3.0. Phylogenetic tree was constructed using MEGA7 and docking of regulatory elements with the corresponding Transcription factors was performed using pyDockDNA. The *Os*GLP12-*3* promoter region (1863bp) was PCR amplified using genomic DNA from Indica rice cultivar Dilrosh-97 and subsequently purified and sequenced. Sequence analysis using Plant Care online database revealed 32 different regulatory elements in Nipponbare while 31 different regulatory elements were found in Dilrosh-97. The cis-acting elements were found to be involved in plant hormonal inductions, developments and biotic and abiotic stress response. Notably, the *Os*GLP12-*3* gene promoter from Dilrosh-97 was found to contain higher number of TATA-box and CAAT-box as compared to Nipponbare, reflecting it to be efficient in transcription initiation. Pairwise alignment using NCBI Nucleotide Blast tool showed 99% similarity between subject sequence (Nipponbare) and query sequence (Dilrosh-97). Localization of Transcription factors motif sequences indicate that the motifs including Homeodomain; TALE, NF-YB and GGAAAt in Nipponbare promoter and Alpha-amylase and B3 in Dilrosh-97 promoter were observed abundantly close to the transcriptional initiation site. Expression analysis using TENOR identified that *Os*GLP12-*3* gene show high to minimal level of expression in response to different stress stimuli. Phylogenetic analysis of the Dilrosh-97 promoter sequence showed close relationship of this promoter with the Japonica rice cultivar Nipponbare. Three transcription factors including B3, GT-1 and NF-YB were subjected to docking with their corresponding cis-acting elements regulatory elements using pyDockDNA to evaluate the nature of interaction between the proteins and DNA sequences. The docking analysis revealed the hydrogen bonding interaction between cis-acting elements and their corresponding Transcription factors.

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Introduction

Half of the world's population depends on rice as a basic food, contributing more than 20% of calories consumed globally, in West Indies, South Asia, Latin America, Middle East particularly (Fukagawa and Ziska, 2019). Rice is grown in more than 100 countries with worldwide production of 645 million tons, of which 90% is contributed by Asian farmers (Sharif *et al*., 2014). In rice production Pakistan ranks 11th globally and share 7% of the total global market, and 3rd in the rice exporter which is the main source of foreign revenue income (Shah *et al*., 2020). The production of rice is highly affected by biotic and abiotic stresses such as drought, salinity, diseases, insects and heat due to climate change, decline of water and change in seasonal pattern due to global warming. These major challenges can be solved by identifying the genes and their products to deal with such adverse situations.

Numerous genes have been identified in different plants which express in response to different stress stimuli, among which, Germins and Germins-like proteins (GLPs) are abundantly found in different plants. Germins are from the cupin superfamily of proteins and existence of these proteins has been reported in different plants, likes dicots, monocot, slime mold and gymnosperms (Ali and Mahmood, 2015). Because of enzymatic activities the Germins and GLPs play a major role in plant defense against pathogens and abiotic stresses directly or indirectly (Durrani *et al*., 2019).

In plants GLPs are involved in different biochemical, enzymatic and physiological activities. Enzymatic activities such as superoxide dismutase (SOD), AGPPase and oxalate oxidase (OXO) have been linked with several GLPs. Hydrogen peroxide (H_2O_2) generation by superoxide dismutase (SOD) and oxalate oxidase (OXO) activities contributed by GLPs has been demonstrated to play important role in disease resistance in plants as well the higher level of H_2O_2, induces structural modification in plant cell wall which leads to the programed cell death (Chattopadhyay, 2014). The rice *Os*GLP1 a cell wall localized protein having SOD activity has been reported to be involved in defense against fungal pathogen attack (Banerjee *et al*., 2010). The GLPs have been extensively studied in rice due to their diverse biochemical, enzymatic and physiological role against biotic and abiotic stresses.

Salinity and drought are the major abiotic stress factors that specifically effect the yield of rice (Shankar *et al*., 2016). In light of these phenomenon, the study of the stress-responsive GLP genes can be useful for identification of stress tolerance elements in the genomes of different plant species. So far, through genome sequencing numerous GLPs genes have been found in plants such as barely, rice and Arabidopsis (Wu *et al*., 2000). About 43 Germin like proteins were recently discovered in rice, and the majority of them were found to be located in the extracellular region, more precisely in the cell wall. The genes for these GLPs were reported to be located on chromosomes 1, 2, 3, 4, 5, 8, 9, 11 and 12 however, on chromosome 3 and 8 highest number of *Os*GLP genes were found to exist as a small cluster (Anum *et al*., 2022). As the promoter governs the regulated expression of their downstream genes, therefore, *Os*GLP2 promoter has been found to be involved in wound inducible activities as well as abiotic stress responses including salinity and drought (Mahmood *et al*., 2013). Additionally, identification and analysis of the regulatory elements, present in the promoter region of GLPs genes, is an efficient approach towards understanding of gene expression against a particular stress stimulus.

A segment known as a gene promoter region, which is located typically 1000 base pairs upstream of the transcriptional initiation site, controls the initiation and regulation of a gene's transcription. (Dean and Schmidt, 1995). The cis-acting elements or transcription factor binding site reside in the promoter region which is involved in spatiotemporal gene expression (Yamamoto *et al*., 2007). In plant frequently studied regulatory elements against biotic and abiotic stress response, include NAC, WRKY, BHLH, bZIP, NAM/ATAF/CUC2, APETALA and MYB, C2H2, Zinc finger protein and MADS (Pandey and Somssich 2009; Schwechheimer *et al*., 1998).

The NAC cis-acting elements has been found in different *Os*GLP gene promoters, and were found to be activated in response to biotic/abiotic stress signals. Expression analysis of NAC gene through microarray reveal that 45 NAC genes were upregulated in rice against biotic stresses. Moreover, about 26 NAC genes were distinctively expressed against rice stripe virus and rice tungo spherical virus (Nuruzzaman *et*

al., 2010). Enhanced tolerance against dehydration and salinity was reported due to overexpression of NAC in rice (Hu *et al*., 2006). To further unravel the biological importance of these cis-acting regulatory elements *In Silico* analysis provide a novel approach and have many applications in crop biotechnology.

Modern bioinformatics tools such as, NCBI Blast, PlantPAN 3.0 (Chang *et al*., 2008), PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/htmll), molecular evolution genetic analysis 7 (MEGA7) (Yamamoto *et al*., 2007), rice genome annotation project database (http://rice.uga.edu/), and clustal W provide in-depth approaches to investigate various properties of proteins, genes and their promoters. The *In Silico* analyses are cost effective and valuable techniques for functional characterization of genes and have potential uses in transgenic techniques, therefore the current study was aimed on promoter analysis of *Os*GLP12-3 gene from rice cultivar Dilrosh 97. Promoter analysis helps identify cis acting elements that are the binding sites of transcription factors. The cis acting elements are under strict transcriptional regulation. Their corresponding transcription factor's expression is induced by biotic and abiotic environmental factors which regulate the downstream gene's expression in response to concerned factors and enable the host plants to respond appropriately to abiotic and biotic factors. The rationale of current study was to search for a promoter that could respond to stressful conditions and express the downstream gene to combat stressful conditions.

Therefore, the main focus of the current study was isolation, sequencing and in silico investigation of *OsGLP12-3* gene promoter from rice cultivar Dillrosh-97.

Materials and Methods

Plant material

The rice cultivar Dilrosh 97 seeds were generously provided by Dr. Israr Ud Din from Plant Tissue Culture and Germplasm Conservation Lab-2, at Institute of Biotechnology and Genetic Engineering Peshawar, Pakistan.

Sterilization and germination

Mature and healthy seeds were de-husked physically taking care not to damage the embryos. Seeds were then washed with tap water properly. After washing seeds were soaked in bleach solution (20% bleach and 80% water) for 3 to 4 minutes with continuous shaking. Seeds were removed from the bleach solution and rinsed five times with sterile double distilled water to completely remove the bleach. Finally, the seeds were dried using sterile filter paper and germinated hydroponically under illuminated conditions with approximately 50% humidity, on sterile MS media and the fresh leaves were taken for further DNA isolation.

DNA isolation

From fresh and healthy leaves DNA was isolated through CTAB method as described by (Richards *et al*., 1994), with slight modification, and stored at -20 ˚C for further use.

Oligo design

Pair of oligos was designed manually, on the chromosome 12 BAC clone of *Oryza sativa* cv Nipponbare, (GenBank accession number: AP014968.1) to amplify the promoter region of *Os*GLP12-3 gene from Oryza sativa cultivar Dilrosh-97 as shown in Table 1. The properties of primers were checked using Integrated DNA Technology Oligo analyzer tool available at https://eu.idtdna.com/calc/analyzer (Dean and Schmidt, 1995).

PCR amplification of OsGLP12-3 promoter

The oligo pair as mentioned in Table 1 was used to perform a 100 µl polymerase chain reaction for the amplification of *Os*GLP12-3 gene promoter from Indica rice cultivar Dilrosh-97. The reaction mixture comprised of molecular biology products high fidelity hot start PCR master mix cat# G014-Dye 1X, forward and reverse oligos 0.125 Pico moles and 1 µg DNA. The reactants were subject to thermal cycling for 40 times with initial denaturation at 94 °C for three minutes, denaturation at 94 °C for thirty seconds, annealing at 51 °C for 30 seconds, extension at 72 °C for 1 min and 40 seconds. After completion of thermal cycling reaction mix was subjected to further final extension at 72 °C for 7 minutes.

Gel electrophoresis and gel documentation

The amplified product was resolved on 1% TBE gel, at 110V for 40 minutes. The gel was visualized by Ethidium bromide UV trans-illumination by using UV Tech, United Kingdom, Gel documentation system. The "Thermo Scientific Gene Ruler" 1kb plus DNA ladder was used as marker for identification of desired amplicon.

Table 1: *Sequences of the primers used for the amplification of OsGLP12-3 gene promoter from Indica rice cultivar Dilrosh-97.*

Oligo	Sequence
*Os*GLP12-3 Sense Primer	5'- CACC ACC TTG ACT TGT TGT CAG -3'
*Os*GLP12-3 Antisense Primer	5'- CAT GTT AAG TTG ATG GA ACT TTT G -3'

Amplicon purification and sequencing
The amplified PCR product was subject to purification and sequencing by acquiring services from BGI China.

Pairwise alignment
Pairwise alignment was performed using NCBI Nucleotide BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The *Os*GLP12-3 promoter sequence from Nipponbare was retrieved from NCBI public database and was used as subject sequence for pairwise alignment with *Os*GLP12-3 promoter sequence from rice cultivar Dilrosh-97, using as query sequence.

Multiple sequence alignment and phylogenetic analysis
For multiple sequence alignment of *Os*GLP12-3 promoter, homologous GLP promoter sequences from other plants were retrieved from NCBI nucleotide BLAST database in FASTA format. The retrieved sequences were imported into MEGA 7 program and were subsequently subjected to multiple sequence alignment by opting MUSCLE. The MEGA 7 program was also exploited for construction of phylogenetic tree, through Neighbor joining method, in order to determine evolutionary relationship between *Os*GLP12-3 promoter and homologous promoters from other GLPs.

Identification and mapping of cis acting regulatory elements
The cis acting regulatory elements *Os*GLP12-3 promoter from rice cultivars Nipponbare and Dilrosh 97 were searched using online program PlantCARE available at (http://bioinformatics.psb.ugent.be/webtools/plantcare/html). Map of transcription factor binding sites (cis acting elements) and their corresponding factors was constructed using online program Plant PAN 3.0 database, available at plantpan.itps.ncku.edu.tw (Chang *et al.*, 2008).

Expression analysis
Transcriptome data of *Os*GLP12-3 gene was retrieved from TENOR database available at (http://tenor.dna.affrc.go.jp/) to predict gene expression during development and also in response to different biotic and abiotic stress conditions.

Molecular modelling
For modelling cis-acting elements, Discovery Studio Visualizer was used which provides 3D structure of DNA in PDB format. From the protein data bank (PDB) (http://www.rcsb.org/pdb) the 3D structures of transcription factors were obtained (Berman *et al.*, 2000). The transcription factor PDBs were selected on basis of amino acids length, missing residues, atomic resolution, mutation and structure type, and were used for further protein- DNA docking.

Molecular docking and visualization
DNA-Protein docking was performed using an online available web base service pyDockDNA available at (https://model3dbio.csic.es/pydockdna) (Rodríguez-Lumbreras *et al.*, 2022) and discovery studio visualizer was used for graphical analysis of all the models.

Results and Discussion

The *OsGLP12-3* promoter region, 1863 bp was PCR amplified using high fidelity Taq polymerase and resolved on 1% TBE agarose gel and finally subject to gel documentation analysis, using ethidium bromide UV transillumination as shown in Figure 1.

Pairwise alignment
For pairwise alignment OsGLP12-3 gene's promoter sequence from rice cultivar Dilrosh-97 was used as query sequence for alignment with same gene's promoter sequence from rice cultivar Nipponbare, wherein the later sequence was used as subject sequence. Pairwise alignment revealed 99 % similarity between query and subject sequences as shown in Figure 2.

Multiple sequence alignment and phylogenetic analysis
Multiple sequence alignment and phylogenetic analysis were performed to determine the evolutionary relationship among *Os*GLP12-3 promoter region from

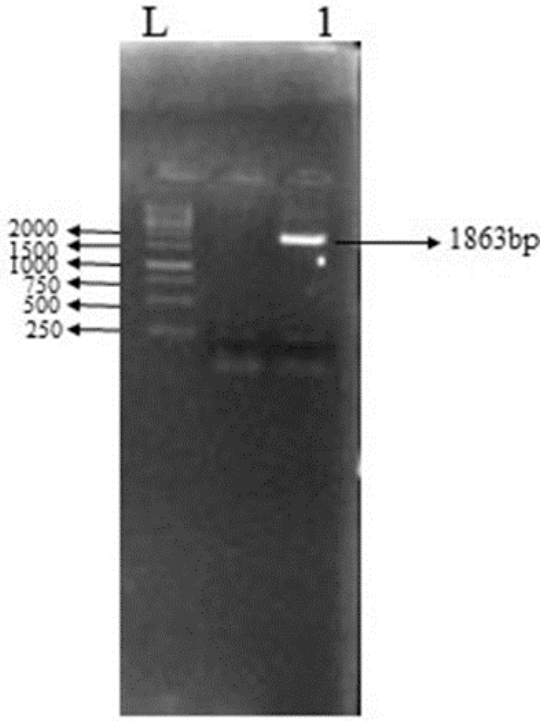

Figure 1: *PCR Amplification of OsGLP12-3 Promoter region from Indica rice cultivar Dilrosh-97.*
Lane L indicate 1KB+ DNA Marker and Lane 1 represents the amplified OsGLP12-3 Promoter region from Dilrosh 97.

rice cultivar Dilrosh 97, and *Os*GLP12-3 promoters from other plants. Phylogenetic analysis included construction of phylogenetic tree through neighbor joining method and confidence level was evaluated with 1000 bootstraps. Phylogenetic analysis resulted in the formation of two clades I and II. Clade I was found to contain 10 sequences while clade II contained 4 sequences. According to phylogenetic tree as shown in Figure 3, clade I can be further divided into two clusters, of which cluster 1 contains 6 sequences including Oryza sativa cultivar Swat 1 *Os*GLP12-3, Oryza Sativa Japonica cultivar CP01142Chanxianggeng 1813, Oryza sativa Indica cultivar RP-Bio 226, Oryza sativa Japonica cultivar Nipponbare, CP101145 Chanxianggeng 1813 and Oryza sativa Cultivar Basmati, 2008) and cluster 2 contained 4 sequences including Oryza Sativa Japonica Nipponbare, Oryza sativa cultivar Dilrosh-97 *GLP12-3* gene's promoter, Oryza Sativa Indica Shuhui 498. Whereas Clade II contained 4 sequences from Oryza sativa Indica Zhenshan-97, Oryza sativa Indica Minghui 63, Oryza sativa Japonica Nipponbare, and Oryza sativa OSJNBa. Generally, Oryza sativa Japonica Nipponbare *Os*GLP12-3 promoter was identified closely related to Indica group cultivar Dilrosh-97 *OsGLP12-3* gene promoter as shown in Figure 3.

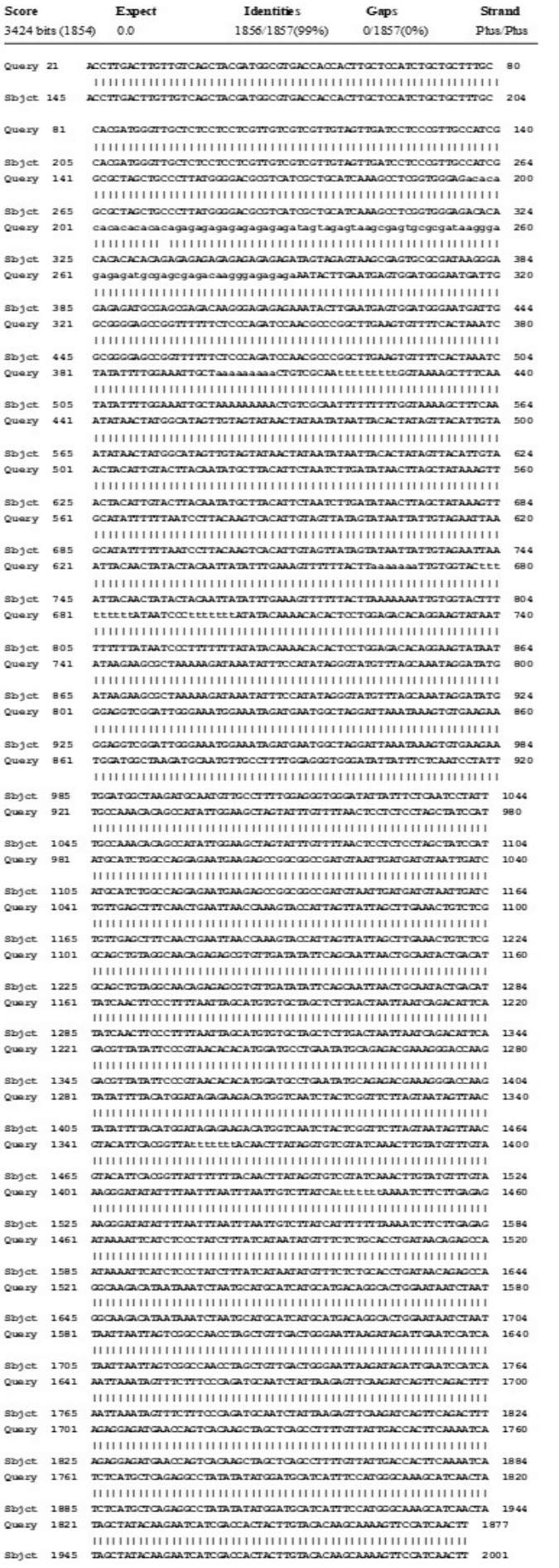

Figure 2: *Pairwise Alignment showing 99% similarity between Nipponbare and Dilrosh-97 OsGLP12-3 promoter sequence.*

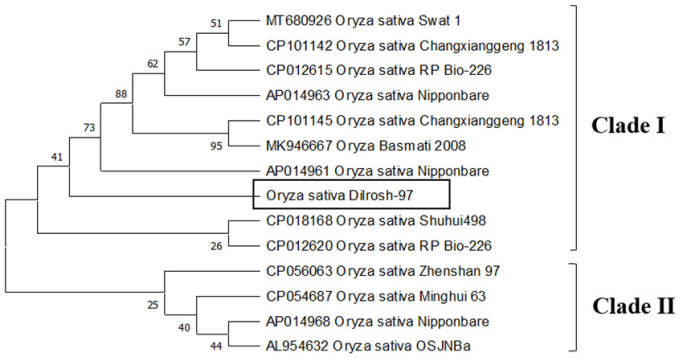

Figure 3: *Phylogenetic tree showing evolutionary relationship between OsGLP12-3 promoter from Dilrosh 97 and other Germin Like Protein Promoters.*

Description and analysis of important regulatory elements

In *OsGLP12-3* promoter region from Nipponbare and Dilrosh-97 different cis-acting regulatory elements were found. Of all cis-acting elements a total of 32 important elements with their proposed functions from both cultivars (Dilrosh 97 and Nipponbare) were selected and found to be distributed, at variable locations and with variable frequencies, throughout the promoter region on both strands as shown in Table 2.

Specific elements with their proposed functions

Light-responsive elements: Plant Care analysis in both rice cultivars found that except ATCT-motif, single copy of different light responsive elements including Box-4, GATA-motif, LAMAP-element, I-Box, GT1-motif, and chs-CMA1a were identified in the *OsGLP12-3* promoter with enormous sequence variation. This reflects that expression of *Os*GLP12-3 gene is induced by light in both cultivars.

Table 2: *Comparative analysis of cis-acting regulatory elements in the OsGLP12-3 promoter region from Nipponbare and Dilrosh-97. V1 and V2 represents Nipponbare and Dilrosh-97 Varieties, respectively.*

S. No	Name of the regulatory element	The sequence of the element	Copy number		Location		The function of regulatory element
			V1	V2	V1	V2	
1	O2-site	GATGACATG-G	1	1	1423	568	Involved in zein metabolism regulation
2	RY-element	CATGCATG	1	1	1674	319	Involved in seed-specific regulation
3	DRE core	GCCGAC	1	1	1714	281	Not reported
4	TCCC-motif	TCTCCCT	3	3	379,403,1594	400, 1591, 1615	Part of the light responsive element
5	Box-4	ATTAAT	3	3	1328,1702,1706	289, 293, 66	Part of a conserved DNA module involved in light responsiveness
6	GATA-motif	GATAGGG	1	1	1597	397	Part of light responsive element
7	TATC-Box	TATCCCA	1	1	1019	975	Involved in gibberellin – responsiveness
8	LAMAP-element	CTTTATCA	1	1	1603	390	Part of light responsive element
9	TGA-element	AACGAC	1	1	233	1762	Auxin –responsive element
10	I-Box	TGATAATGT, CATATCCAAT	2	2	914,1280	712, 1076	Involved in light response
11	TATA-Box	TATACA, TATA, ATATAT, TATAAGAA, TACAAAA, ATTATA, TATAA,	51	55	45, 46, 47, 504, 564, 565, 587, 593, 597, 598, 609, 663, 664, 675, 718, 719, 724, 752, 761, 761-763, 806-809, 821-824, 826, 828, 858, 862.1252, 1253, 1348, 1349, 1404, 1491, 1492, 1529, 1530, 1901-1905, 1942, 1948	47, 49, 55, 91-94, 96, 466, 467, 505, 593, 647, 648, 743, 744, 1102, 1130, 1133, 1134, 1137, 1138, 1139, 1166, 1169, 1171-1173, 1186-118, 1233, 1234, 1245, 1271-1273, 1278, 1320-1322, 1333, 1338, 1397-1399, 1402-1404, 1409, 1410, 1431, 1432, 1493	Core promoter element involved around-30 of transcription start

Tables continued on next pages.....

S. No	Name of the regulatory element	The sequence of the element	Copy number		Location		The function of regulatory element
			V1	V2	V1	V2	
12	CGT-CA-motif	CGTCA	1	1	288	1708	Cis-regulatory element involved in the MeJA-responsiveness
13	P-Box	CCTTTTG	2	2	1007,1856	138,987	Involved in gibberellin responsiveness
14	MYB-like sequence	TAACCA	1	1	1184	811	Not reported
15	AT~TA-TA-box	TATATA	5	3	45, 822, 824, 1901, 1903,	92,94,1171	Not reported
16	MYB	CAACAG, TAAC-CA, CAACTG, TAACTG	7	6	72, 1163, 1184, 1235, 1175, 1266, 1728	267,760,811,832	Not reported
17	W-box	TTGACC	2	2	1431,1866	129,564	Binding site for WRKY/ Fungal elicitor-responsive element
18	ATCT-motif	AATCTAATCC	1	2	1658	333	Part of conserved DNA module involved in light responsiveness
19	MYB recog-nition site	CCGTTG	1	1	252	1743	Not reported
20	GARE motif	TCTGTTG	2	2	1162,1235	759,832	Gibberellin-responsive element
21	AA-GAA-motif	GTAAAGAAA, GAAAGAA	3	2	129,1774,1775	217,219	Not reported
22	TGACG motif	TGACG	1	1	288	1708	Element involved in the MeJA-responsiveness
23	MYC	CATGTG	2	2	1306,1364	631,689	Not reported
24	CCAAT-Box	CAACGG	1	1	252	1743	MYBHv1 binding sites
25	GT1-motif	GGTTAAT	2	1	1182,1183	812	Light responsive element
26	as-1	GACG	1	1	288	1708	Not reported
27	CAAT-box	CAAT, CAAAT, CAAAT, CCAAT,	31	32	39,110,440,516,537,561,618,629,639,711,731, 759,766,791,911,933,999,1034,1041,1060,1073,1145,1175,1262,1272,1434,1549,1751,1762, 1789,1865	132,208,234,246,448,563,725,735,840,852,923,936,937, 955,963,998,1063,1064,1085,1206,1230,1238,1266,1286,1358,1368,1379,1435,1460,1481,1556,1557	Common cis-acting element in promoter and enhancer regions
28	ATC-motif	AGCTATCCA	2	1	1094	898	Part of conserved DNA module involved in light responsiveness
29	chs-CMA1a	TTACTTAA	1	1	779	1214	Part of light response element
30	MBS	CAACTG	1	1	1175	820	MYB binding site involved in drought inducibility
31	ERE	ATTTTAAA	2	1	16,1565	428	Ethylene-responsive elements
32	WUN-motif	CAATTACAT	2	0	1140, 1152	0	Not reported

Hormonal responsive elements

According to promoter analysis results in current study, three the Gibberellin response elements TATC-Box, P-Box and GARE-motif, were identified in the *OsGLP12-3* promoter region. Single copy of TATC-box and two copies of P-Box, GARE-

motif (TCTGTTG) were observed in Nipponbare and Dilrosh-97. Two Methyl Jasmonate responsive elements CGTCA, TGACG were observed and single copy of CGTCA and TGACG was found in both rice cultivars. Ethylene-responsive elements (Angarica *et al.*, 2008) having a sequence (ATTTTAAA) were also identified, with two copies of ERE in Nipponbare and single copy in Dilrosh-97.

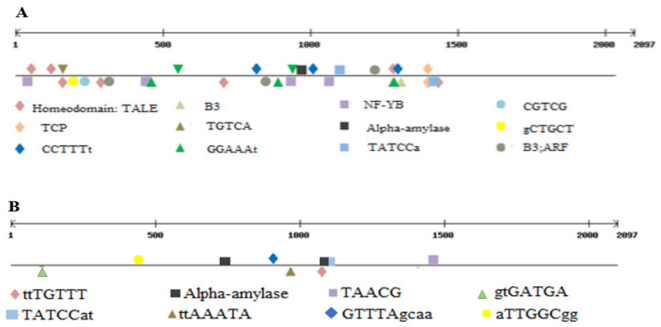

Figure 4: *A, B: Representative map showing distribution and localization of selected transcription factor binding sites within 1.5 Kb OsGLP12-3 promoter region from Nipponbare.*
Legend: Upper region of line represents Plus Strand; Lower region of line represents Minus strand.

Seed-specific elements

During analysis, seed-specific RY-element (CATGCATG) was found with a single copy number in the promoter of Nipponbare and Dilrosh-97. The TATA boxes with variable sequences and copy number were observed in both rice cultivars. Nipponbare revealed 51 copies of TATA boxes as compared to Dilrosh-97 where 55 copies of TATA box were identified.

Similarly, the position and copy number of CAAT box also varied among both rice cultivars, 31 copies of CAAT box were identified in Nipponbare whereas Dilrosh-97 revealed 32 copies of CAAT box. Both TATA box and CAAT box are involved in transcription initiation, therefore it can be said that *Os*GLP12-3 gene's promoter from Dilrosh 97 is more powerful in context of transcription initiation.

Stress and defense responsive elements

With variation in copy number and position, *OsGLP12-3* promoter region contained stress and defense response elements. These elements are involved in gene expression during drought and defense response. Single copy of drought response CAACTG at different position (1175-Nipponbare and 820-Dilrosh-97) was found in both cultivars. Two copies of W-box elements were found at different position in Nipponbare and Dilrosh-97. The W-box cis-acting element is the binding site of WRKY transcription factors which is involved in biotic and abiotic stress responses in plants.

Mapping of regulatory elements

The transcription factor binding sites having important functions were mapped within 1.5kb promoter region from both rice cultivars including Nipponbare and Dilrosh-97, based on high similar score using web-based service PlantPAN3.0 as shown in Figure 5A, B and 6C, D.

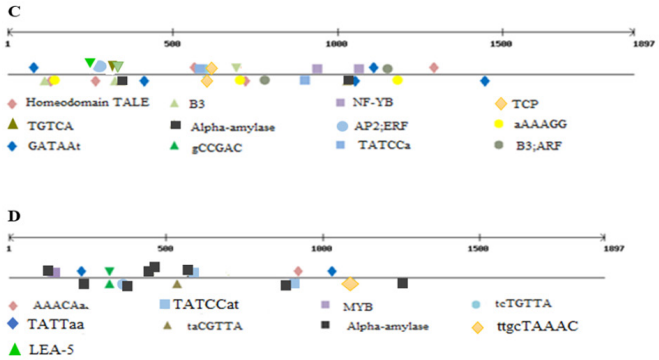

Figure 5: *C, D: Representative map showing distribution and localization of selected transcription factor binding sites within 1.5 Kb OsGLP12-3 promoter region from Dilrosh- 97.*
Legend: Upper region of line represents Plus Strand; Lower region of line represents Minus strand.

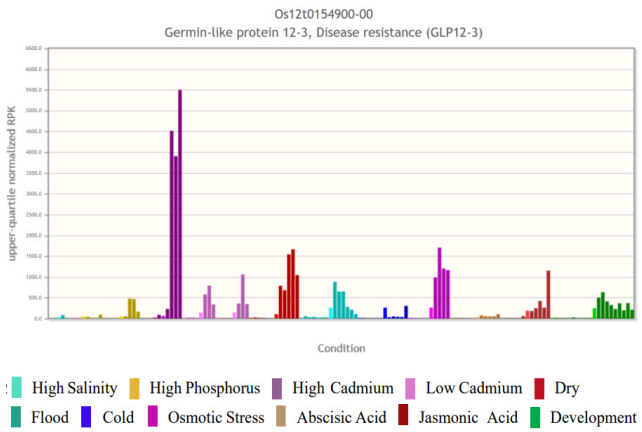

Figure 6: *Expression profile of OsGLP12-3 gene during development and induction by hormones and different stress conditions. Adopted from (Durrani et al., 2019).*

In silico OsGLP12-3 gene expression analysis in rice

In Silico expression analysis was performed usig TENOR database as described by Durrani *et al.*, (2019). Transcriptomic data of *Os*GLP12-3 gene, retrieved from TENOR database, reveals that *Os*GLP12-3 gene expresses predominantly in roots. Expression analysis included expression during development and expression in response to different abiotic and biotic stress stimuli as shown in Figure 7.

Table 3: *pyDockDNA web server results of interactions between transcription factors and cis-acting elements. The pyDockDNA score is a combination of Van der Waals, electrostatic, and desolvation.*

Proteins/DNA	Configuration	pyDockDNA score (l/Mol)	Van der Waals Energy (Kcal/mol) (Kcal/Mol)	Electrostatic energy (Kcal/mol)	Desolvation energy (Kcal/mol)
B3- GCATG	4849	-43.527	13.506	-66.042	21.164
B3- CATGCATT	5778	-62.249	-19.791	-80.515	20.245
GT-1- GGAAAt	6017	-54.154	8.086	-68.588	13.457
GT-1- GATAATGTC	1673	-59.947	27.296	-62.311	-0.393
NF-YB- CCAAT	5794	-41.239	-1.216	-62.800	21.683
NF-YB- ATCGGCC	1184	-47.740	-26.508	-62.456	17.367

Table 4: *Possible hydrogen bonds between Transcription factors and corresponding DNA sequences.*

Possible hydrogen bonds between B3 and DNA sequence GCATG						
Protein residue	**Protein atoms**	**Amino acid position**	**DNA residue**	**DNA atoms**	**Nucleotide position**	**Distance in Å**
LYS	NZ	101	A	O3	3	2.44
THR	OG1	100	G	O1P	5	3.13
SER	OG	89	G	O3	5	3.35
TYR	O	90	G	O3	5	3.17
ARG	NE	105	T	O1P	3	2.01
ARG	NH2	85	T	O3	3	3.16
Possible hydrogen bonds between GT-1 and DNA sequence GGAAAt						
HIS	NE2	151	A	N6	3	2.92
LYS	O	150	A	N6	4	2.76
LYS	O	150	A	N6	5	3.23
ALA	O	149	A	N6	4	1.89
ALA	O	149	A	N6	3	3.29
LYS	O	147	A	N6	4	2.39
PHE	O	146	A	N6	5	1.93
ARG	NE	100	T	O3	6	2.79
Possible hydrogen bonds between GT-1 and DNA sequence GATAATGTC						
LYS	NZ	82	A	N3	4	3.29
LYS	N	81	A	O3	5	3.11
Possible hydrogen bonds between NF-YB and DNA sequence CCAAT						
ARG	NE	112	T	O3	5	2.68
ILE	N	51	A	O1P	4	3.24
ILE	N	51	A	02P	4	3.0
SER	OG	38	C	O3	2	2.51
SER	OG	38	C	O2P	2	3.08
ARG	NH1	39	C	O3	1	1.67
Possible hydrogen bonds between NF-YB and DNA sequence ATCGGCC						
TRY	OH	110	C	O3	7	1.43
HIS	ND1	69	C	O1P	7	2.35
LEU	O	71	C	N4	7	2.23
SER	O	70	C	N4	6	3.32
SER	OG	70	G	O1P	5	3.13

Interaction of transcription factors with regulatory elements (Docking)

Protein-DNA interaction play an important role in DNA modification and gene regulation, so analyzing this interaction is of great value (Qin and Zhou, 2011). In current research, three transcription factors including B3, GT-1 and NF-YB were identified for binding to their corresponding regulatory elements in the promoter region of Dilrosh-97 using pyDockDNA program. The interaction between transcription factors and their corresponding regulatory elements (Docking analysis) revealed hydrogen bonding interactions as reported in previous studies (Angarica *et al*., 2008). The results obtained from pyDockDNA webserver are given in Table 3.

Analysis of protein -DNA docking finding

In order to have better understanding regarding the transcriptional regulation of *OsGLP12-3* gene expression in the current study three transcription factors including B3, GT-1 and NF-YB were subjected modeling and simultaneously their corresponding DNA elements were also modeled using discovery studio program. These Transcription factors were found abundantly and play important regulatory role in plants.

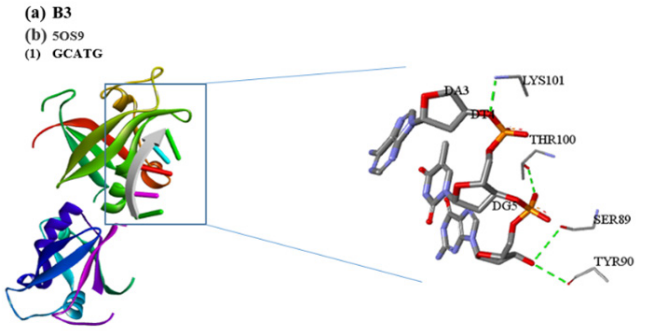

Figure 7: *Interaction between B3 protein (TF) with DNA sequence GCATG.*

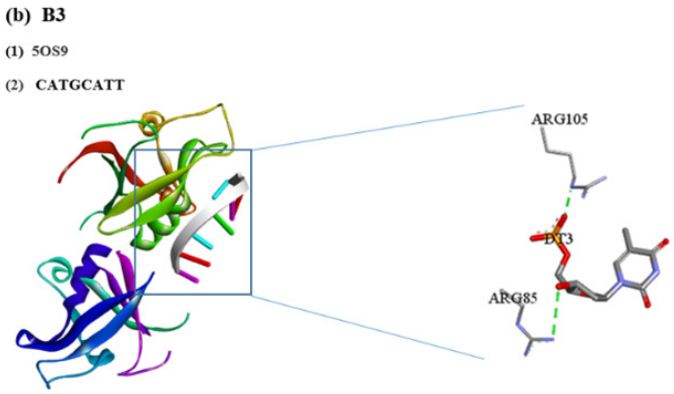

Figure 8: *Interaction between B3 protein (TF) with DNA sequence CATGCATT.*

By making hydrogen bonds the Transcription factors interact with regulatory element as shown by blue dots in the representative structure models of all complexes (Figures 7, 8, 9, 10, 11, 12). The detail of hydrogen bonding between the residues and corresponding regulatory elements are summarized in Table 4.

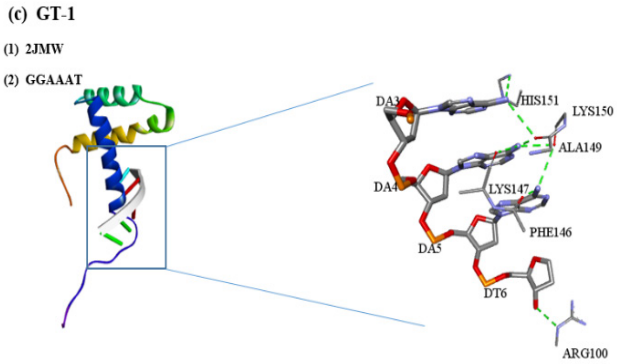

Figure 9: *Interaction between GT-1 protein (TF) with DNA sequence GGAAAt.*

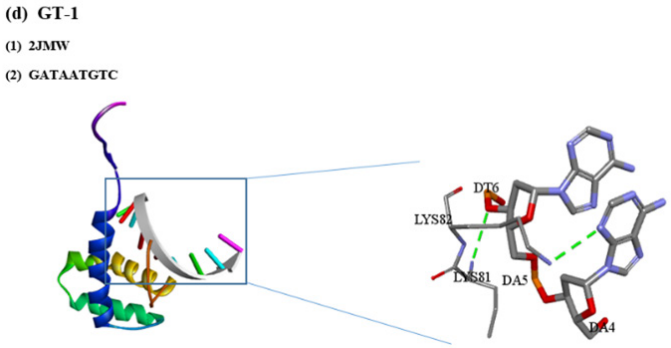

Figure 10: *Interaction between GT-1 protein (TF) with DNA sequence GATAATGTC.*

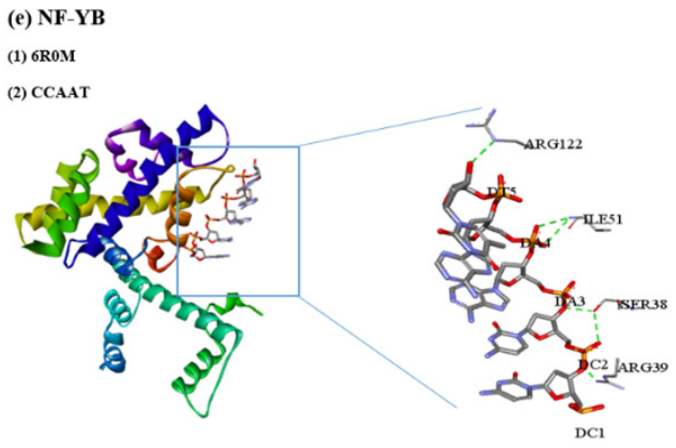

Figure 11: *Interaction between NF-YB protein (TF) with DNA sequence CCAAT.*

The transcription factor B3 was docked with its corresponding cis-acting elements including 5'-CGATG-3' and 5'-CATGCATT-3'. The docking analysis revealed that B3 when docked with the element 5'-CGATG-3', it formed strong hydrogen

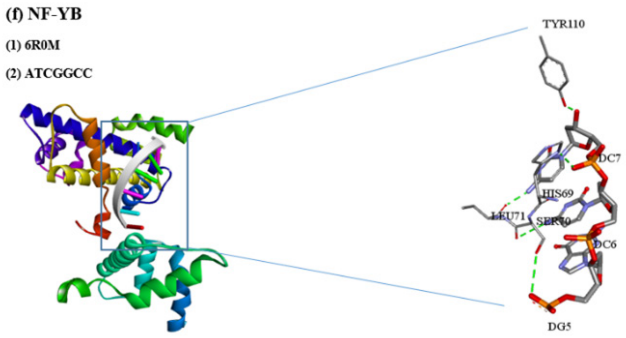

Figure 12: *Interaction between NF-YB protein (TF) with DNA sequence ATCGGCC.*

bonds between LYS101 (NZ) and O3. Furthermore, hydrogen bonding was also observed for THR100 (OG1) with O1P, SER (Richards *et al.*,) with O3 and TYR (Fukagawa and Ziska) with O3. While B3 when subjected to docking with element 5'-CATGCATT-3', ARG105 formed a strong bond with O1P followed by ARG85 (NH2) with O3. Another Transcription factors- GT-1 was subjected to docking with regulatory elements 5'-GGAAAt-3' and 5'-GATAATGTC-3'. Again, transcription factor GT-1 when docked with GGAAAt, revealed strong hydrogen bonds for ALA149 (Fukagawa and Ziska) with N6 atom of fourth nucleotide followed by PHE146 (Fukagawa and Ziska) with N6 and vis versa. Moreover, when transcription factor GT-1 was docked with element GATAATGTC, LYS82 (NZ), it formed bond with N3 and LYS81 (Fukagawa and Ziska) with O3. Also, NF-YB Transcription factors was subjected to docking with elements 5'-CCAAT-3' and 5'-ATCGGCC-3'. NF-YB when docked with element CCAAT, SER38 (Richards *et al.*, 1994) formed strongest hydrogen bond with O3 followed by ARG39 (NH1) with O3. Moreover, a ILE51 and SER38 formed a double hydrogen bond while NF-YB was subject to docking with ATCGGCC, strong hydrogen bonding was observed between TRY100 (Nuruzzaman *et al.*, 2010) with O3 followed by LEU71 (Fukagawa and Ziska, 2019) with N4 in this case.

The extreme change in climatic patterns and increase in global warning has caused a major adverse effect on crops worldwide in the 21st century (Rosenzweig *et al.*, 2014). Rice (*Oryza sativa L.*) contributes more than 20% of calories consumed worldwide and has become a basic food for half of world's population (Fukagawa and Ziska, 2019) but the production of rice has been adversely effected by different environmental stresses, particularly drought and salinity. Different stress resistance rice cultivars have been reported over the years (Lenka *et al.*, 2011). Previously, 30 GLP genes in roots from japonica rice cultivar Nipponbare has been sequenced and studied (Mahmood *et al.*, 2010). Notably, the japonica group cultivar Nipponbare genome has been completely sequenced and its sequence is available on public database.

Germins and Germin-like proteins (GLPs) are the most diverse and ubiquitous proteins that belong to the cupin superfamily. From previous studies it is evident that germins and GLP play an important role in response to stresses both biotic and abiotic. Moreover, in plant defense systems and development pathways Germins and GLPs has been found to play a major role (Bernier and Berna, 2001).

Sequence similarity analysis

In the current study 99% similarity has been found between *Os*GLP12-3 promoter sequences from rice cultivars Nipponbare and Dilrosh-97 reflecting that both cultivars are very much similar in the genomic DNA sequence spanning *OsGLP12-3* promoter.

Phylogenetic analysis

Also, in the current study a variable homology was found by phylogenetic analysis among the nucleotide sequences of different GLPs from different rice cultivars. Previous studies suggest that *Os*GLPs localized on the same chromosome show closer relationship to each other (Ilyas *et al.*, 2020). Similar results were also observed in our phylogram where the promoter sequences from GLPs localized on chromosome 12, were clustered together in the same cluster and similar behavior of presence of promoter sequences, in a single cluster, from GLPs localized on chromosome 5 was observed. According to Lu *et al.* (2010) Germin and GLPs genes might be evolved through independent gene duplication events. In our results, variability among the *OsGLP12-3* and other GLPs might be due to the same events.

Expression profile

In a study conducted by Lu *et al.* (2010) on soyabean GLPs, diverse expression pattern under different stress conditions and development has been observed likewise the transcriptome data of OsGLP12-3 gene, retrieved by Durrani *et al.* (2019) also report OsGLP12-3 gene's expression induced by hormones including Abscisic acid and Jasmonic acid. The current investigation identified the cis-acting regulatory

element having sequence TGACG that is recognition site for Methyl Jasmonate (MeJA). Plants produce Jasmonate and methyl Jasmonate in response to may biotic and abiotic stresses particularly herbivory and wounding. In turn these hormones signal the original plant's defense systems. This reflects that Dilrosh-97 have inbuilt defense mechanism against herbivory and wounding owing to presence of TGACG motif in its OsGLP12-3 promoter.

Transcription factor binding sites/ cis-acting regulatory elements

Transcription regulatory elements including I-box, Box-4, G-box, GA-motif and GATA-motif, are the well define light response elements (Ibraheem *et al*., 2010). These light responsive elements have been identified in the regulatory region of light-regulated genes, important for light-driven gene expression (Lam and Chua, 1989). The light response elements not solely confer light response but work synergistically with various elements, making a light responsive unit (López-Ochoa *et al*., 2007). In the present study, several light-response elements (lREs) including ATCT element, Box-4, GATA-motif, LAMAP-element, I-Box, GT1-motif, and chs-CMA1a element were identified in the *OsGLP12-3* promoter region of Nipponbare and Dilrosh-97. Coexistence of these light responsive elements in the *OsGLP12-3* promoter indicate that these elements work synergistically and *Os*GLP12-3 gene expression is majorly induced by light. The GARE motif, P-box, and TATC-box are Gibberellin-responsive elements. The CGTCA-motif, a MeJA-responsive element and Ethylene-responsive elements with varying copy numbers have been reported in SBP-Box Gene's promoter in *Camellia sinensis* (*CsSBP*) (Wang *et al*., 2018). We also hereby report the presence of the above stated elements in both cultivars i.e., Nipponbare and Dilrosh-97.

MeJA can induce the plant to produce multiple different types of defense chemicals such as phytoalexins (antimicrobial), nicotine or protease inhibitors. The protease inhibitors interfere with the insect digestive process and discourage the insect from eating the plant again.

Our analysis also revealed single copy of drought responsive elements (CAACTG) in *Os*GLP12-3 promoter region. A drought responsive cis-acting CAACTG from the wheat TaFTSH6 gene promoter sequence was also discovered recently. Furthermore, two copies of W-box cis acting elements having sequence, "TTGACC", were also revealed in *OsGLP12-3* promoter region of Nipponbare and Dilrosh-97. To regulate gene expression, the WRKY domain binds the W-box elements ((T) TGACC/T) of target genes (Choi, 2015) In plants WRKY transcription factors play an extensive role and regulates hormonal signaling of Salicylic acid, Jasmonic acid and ethylene, which are involved in the development of response against biotic and abiotic stresses (Erpen *et al*., 2018). In rice many studies reported that the WRKY TFs play an important role in defense against pathogen (Chujo *et al*., 2007).

Interaction of transcription factors with corresponding regulatory elements

In many biological activities the DNA-protein interactions are critical as these interactions play an important role in DNA modifications, regulatory functions and gene expression (Qin and Zhou, 2011). Diverse *In Silico* techniques has been used for identification of DNA-protein interactions. Most of the computational techniques focus on predicting the transcription factors based gene regulation (Dey *et al*., 2012). For the identification of protein- DNA complexes systematic docking is much more essential (Setny *et al*., 2012). The stability and specificity of a protein 3D structure is contributed by hydrogen bonds, therefore, it is important to evaluate the hydrogen bonding and for hydrogen bonding in protein-DNA interactions a distance < 3.5 Å was expected to be realistic and stable (Coulocheri *et al*., 2007). Mahmood *et al*. (2015) revealed that few cis-acting elements formed a strong stable bond with corresponding transcription factors and were thought to be mainly involved in PPO gene regulation. Their structure comparison between DNA complexes and different transcription factors shows that when the transcription factors bind to different DNA sequence it can adopt other local structures (hydrogen bonding with variable bond distances), Moreover, binding and stability depends on hydrogen bonds number and distance. In the current investigation the docking results reveal that the interaction of cis-acting elements and transcription factors are stabilized by hydrogen bonds formed between them and the structure analysis showed that the same transcription factors adopt different local structures when the bind to different DNA sequence, and stability and binding depend on the hydrogen bond and distance. The stable

interaction of transcription factors and corresponding cis-acting elements primarily might be responsible for *OsGLP12-3* gene regulation.

Conclusions and Recommendations

The rice cultivar Dilrosh- 97 express high degree of similarity with rice cultivar Nipponbare within the *Os*GLP12-3 promoter region. Phylogenetically both cultivars are close enough with reference to their *Os*GLP12-3 promoter. Promoter from Dilrosh -97 is more potent for use in crop improvement as compared to Nipponbare owing to having a greater number of TATA and CAAT boxes. Stable hydrogen bonds govern the strong interaction of transcription factors with their corresponding elements that is necessary for efficient gene regulation.

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Novelty Statement

The promoter of OSGLP12-3 gene was sequenced and studied for the first time by our team.

Author's Contribution

The study was conducted by Ammar Sohail under the suervision of Dr. Irfan Safdar Durrani. Noreen Asim helped as collaborator of the study.

Conflict of interest
The authors have declared no conflict of interest.

References

Ali, I. and T. Mahmood. 2015. Identification and analysis of regulatory elements in the germin and germin-like proteins family promoters in rice. Turk. J. Bot., 39(3): 389-400. https://doi.org/10.3906/bot-1405-48

Angarica, V.E., A.G. Pérez, A.T. Vasconcelos, J. Collado-Vides and B. Contreras-Moreira. 2008. Prediction of TF target sites based on atomistic models of protein-DNA complexes. BMC Bioinf., 9(1): 1-18. https://doi.org/10.1186/1471-2105-9-436

Anum, J., C. O'Shea, M.Z. Hyder, S. Farrukh, K. Skriver, S.I. Malik and T. Yasmin. 2022. Germin like protein genes exhibit modular expression during salt and drought stress in elite rice cultivars. Mol. Biol. Rep., 49(1): 293-302. https://doi.org/10.1007/s11033-021-06871-3

Banerjee, J., N. Das P. Dey and M.K. Maiti. 2010. Transgenically expressed rice germin-like protein1 in tobacco causes hyper-accumulation of H2O2 and reinforcement of the cell wall components. Biochem. Biophys. Res. Commun., 402(4): 637-643. https://doi.org/10.1016/j.bbrc.2010.10.073

Berman, H., J. Westbrook, Z. Feng, G. Gilliland, T. Bhat, H. Weissig, I. Shindyalov and P. Bourne. 2000. The protein data bank. Nucl. Acids Res., 28: 235–242. https://doi.org/10.1093/nar/28.1.235

Bernier, F. and A. Berna. 2001. Germins and germin-like proteins: Plant do-all proteins. But what do they do exactly? Plant Physiol. Biochem., 39(7-8): 545-554. https://doi.org/10.1016/S0981-9428(01)01285-2

Chang, W.C., T.Y. Lee, H.D. Huang, H.Y. Huang and R.L. Pan. 2008. Plant PAN: Plant promoter analysis navigator, for identifying combinatorial cis-regulatory elements with distance constraint in plant gene groups. BMC Genom., 9(1): 1-14. https://doi.org/10.1186/1471-2164-9-561

Chattopadhyay, T., 2014. *In silico* analysis of the germinlike protein multigene family members of tomato with predicted oxalate oxidase activity. Int. J. Agric., Environ. Biotechnol., 7(4): 669. https://doi.org/10.5958/2230-732X.2014.01374.6

Choi, C., 2015. II; Park, SR; Ahn, I.; Kim, JB; Hwang, DJ Molecular characterization of Oryza sativa WRKY6, which binds to W-box-like element 1 of the Oryza sativa pathogenesis-related (PR) 10a promoter and confers reduced susceptibility to pathogens. New Phytol., 208: 846-859. https://doi.org/10.1111/nph.13516

Chujo, T., R. Takai, C. Akimoto-Tomiyama, S. Ando, E. Minami, Y. Nagamura, H. Kaku, N. Shibuya, M. Yasuda and H. Nakashita. 2007. Involvement of the elicitor-induced gene OsWRKY53 in the expression of defense-related genes in rice. Biochim. Biophys. Acta Gene Struct. Express., 1769(7-8): 497-505. https://doi.org/10.1016/j.bbaexp.2007.04.006

Coulocheri, S.A., D.G. Pigis, K.A. Papavassiliou and A.G. Papavassiliou. 2007. Hydrogen bonds in protein–DNA complexes: Where geometry meets plasticity. Biochimie, 89(11): 1291-1303. https://doi.org/10.1016/j.biochi.2007.07.020

Dean, C. and R. Schmidt. 1995. Plant genomes: A current molecular description. Ann. Rev. Plant Biol., 46(1): 395-418. https://doi.org/10.1146/annurev.pp.46.060195.002143

Dey, B., S. Thukral, S. Krishnan, M. Chakrobarty, S. Gupta, C. Manghani and V. Rani. 2012. DNA–protein interactions: methods for detection and analysis. Mol. Cell. Biochem., 365: 279-299. https://doi.org/10.1007/s11010-012-1269-z

Durrani, I.S., F.I. Butt, A. Jan, N. Asim and S. Naqvi. 2019. *In silico* analysis tools prove basmati 2008 (Indica Rice) to be more meaningful for crop improvement as compared to nipponbare (Japonica rice). Pak. J. Life Soc. Sci., 17 (1)

Erpen, L., H.S. Devi, J.W. Grosser and M. Dutt. 2018. Potential use of the DREB/ERF, MYB, NAC and WRKY transcription factors to improve abiotic and biotic stress in transgenic plants. Plant Cell, Tissue and Organ Culture (PCTOC) 132(1): 1-25. https://doi.org/10.1007/s11240-017-1320-6

Fukagawa, N.K. and L.H. Ziska. 2019. Rice: Importance for global nutrition. J. Nutr. Sci. Vitaminol., 65(Supplement): S2-S3. https://doi.org/10.3177/jnsv.65.S2

Hu, H., M. Dai, J. Yao, B. Xiao, X. Li, Q. Zhang and L. Xiong. 2006. Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc. Natl. Acad. Sci., 103(35): 12987-12992. https://doi.org/10.1073/pnas.0604882103

Ibraheem, O., C.E. Botha and G. Bradley. 2010. *In silico* analysis of cis-acting regulatory elements in 5′ regulatory regions of sucrose transporter gene families in rice (*Oryza sativa* Japonica) and *Arabidopsis thaliana*. Comp. Biol. Chem., 34(5-6): 268-283. https://doi.org/10.1016/j.compbiolchem.2010.09.003

Ilyas, M., m. Irfan, T. Mahmood, H. Hussain and I. Naeem. 2020. Analysis of germin-like protein genes (OsGLPs) family in rice using various in silicoapproaches. Curr. Bioinf., 15(1):17-33. https://doi.org/10.2174/1574893614666190722165130

Lam, E. and Chua, N.H., 1989. ASF-2: A factor that binds to the cauliflower mosaic virus 35S promoter and a conserved GATA motif in Cab promoters. Plant Cell, 1(12): 1147-1156. https://doi.org/10.1105/tpc.1.12.1147

Lenka, S.K., A. Katiyar, V. Chinnusamy and K.C. Bansal. 2011. Comparative analysis of drought-responsive transcriptome in Indica rice genotypes with contrasting drought tolerance. Plant Biotechnol. J., 9(3): 315-327. https://doi.org/10.1111/j.1467-7652.2010.00560.x

López-Ochoa, L., G. Acevedo-Hernández, A. Martínez-Hernández, G. Argüello-Astorga and L. Herrera-Estrella. 2007. Structural relationships between diverse cis-acting elements are critical for the functional properties of a rbcS minimal light regulatory unit. J. Exp. Bot., 58(15-16): 4397-4406. https://doi.org/10.1093/jxb/erm307

Lu, M., Y.P. Han, J.G. Gao, X.J. Wang and W.B. Li. 2010. Identification and analysis of the germin-like gene family in soybean. BMC Genom., 11: 1-15. https://doi.org/10.1186/1471-2164-11-620

Mahmood, T., N. Nazar, T. Yasmin, B.H. Abbasi, M. Ahmad and S.M.S. Naqvi. 2010. Comparative analysis of regulatory elements in different germin-like protein gene promoters. Afr. J. Biotechnol., 9(13): 1871-1880. https://doi.org/10.5897/AJB09.1803

Mahmood, T., M.U. Rehman, E. Aziz, I. Ali and Z.K. Shinwari. 2015. *In silico* analysis, mapping of regulatory elements and corresponding dna-protein interaction in polyphenol oxidase gene promoter from different rice varieties. Pak. J. Bot., 47(6): 2321-2327.

Mahmood, T., T. Yasmin, M. Haque and S. Naqvi. 2013. Characterization of a rice germin-like protein gene promoter. Genet. Mol. Res., 12(1): 360-369. https://doi.org/10.4238/2013.February.7.6

Nuruzzaman, M., R. Manimekalai, A.M. Sharoni, K. Satoh, H. Kondoh, H. Ooka and S. Kikuchi. 2010. Genome-wide analysis of NAC transcription factor family in rice. Gene, 465(1-2): 30-44. https://doi.org/10.1016/j.gene.2010.06.008

Pandey, S.P. and I.E. Somssich. 2009. The role of WRKY transcription factors in plant immunity. Plant Physiol., 150(4): 1648-1655. https://doi.org/10.1104/pp.109.138990

Qin, S. and H.X. Zhou. 2011. Structural models of protein-DNA complexes based on interface

prediction and docking. Curr. Protein Peptide Sci., 12(6): 531-539. https://doi.org/10.2174/138920311796957694

Richards, E., M. Reichardt and S. Rogers. 1994. Preparation of genomic DNA from plant tissue. Curr. Protoc. Mol. Biol., 27(1): 2.3.1-2.3. https://doi.org/10.1002/0471142727.mb0203s27

Rodríguez-Lumbreras, L.A., B. Jiménez-García, S. Giménez-Santamarina and J. Fernández-Recio. 2022. pyDockDNA: A new web server for energy-based protein-DNA docking and scoring. Front. Mol. Biosci., https://doi.org/10.3389/fmolb.2022.988996

Rosenzweig, C., J. Elliott, D. Deryng, A.C. Ruane, C. Müller, A. Arneth, K.J. Boote, C. Folberth, M. Glotter and N. Khabarov. 2014. Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison. Proc. Natl. Acad. Sci., 111(9): 3268-3273. https://doi.org/10.1073/pnas.1222463110

Schwechheimer, C., M. Zourelidou and M. Bevan. 1998. Plant transcription factor studies. Ann. Rev. Plant Biol., 49(1): 127-150. https://doi.org/10.1146/annurev.arplant.49.1.127

Setny, P., R.P. Bahadur and M. Zacharias. 2012. Protein-DNA docking with a coarse-grained force field. BMC Bioinf., 13(1): 1-12. https://doi.org/10.1186/1471-2105-13-228

Shah, B.H., F.S. Hamid, S. Islam, N. Ahmed, F. Ahmad, N. Khan and Q. Zaman. 2020. Evaluation of elite rice (*Oryza sativa* L.) lines for yield and yield components. Pak. J. Agric. Res., 33(1): 135-139. https://doi.org/10.17582/journal.pjar/2020/33.1.135.139

Shankar, R., A. Bhattacharjee and M. Jain. 2016. Transcriptome analysis in different rice cultivars provides novel insights into desiccation and salinity stress responses. Sci. Rep., 6(1): 1-15. https://doi.org/10.1038/srep23719

Sharif, M.K., M.S. Butt, F.M. Anjum and S.H. Khan. 2014. Rice bran: A novel functional ingredient. Crit. Rev. Food Sci. Nutr., 54(6): 807-816. https://doi.org/10.1080/10408398.2011.608586

Wang, P., D. Chen, Y. Zheng, S. Jin, J. Yang and N. Ye. 2018. Identification and expression analyses of SBP-box genes reveal their involvement in abiotic stress and hormone response in tea plant (*Camellia sinensis*). Int. J. Mol. Sci., 19(11): 3404. https://doi.org/10.3390/ijms19113404

Wu, S., A. Druka, H. Horvath, A. Kleinhofs, C.G. Kannangara and D. von Wettstein. 2000. Functional characterization of seed coat-specific members of the barley germin gene family. Plant Physiol. Biochem., 38(9): 685-698. https://doi.org/10.1016/S0981-9428(00)01176-1

Yamamoto, Y.Y., H. Ichida, T. Abe, Y. Suzuki, S. Sugano and J. Obokata. 2007. Differentiation of core promoter architecture between plants and mammals revealed by LDSS analysis. Nucl. Acids Res., 35(18): 6219-6226. https://doi.org/10.1093/nar/gkm685