

Integrating computational and physiological analyses of galactomannan biosynthesis in *Cyamopsis tetragonoloba*

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Abstract

Cyamopsis tetragonoloba (guar or cluster bean) is an important arid legume valued for its medicinal properties and industrial gum production. The principal constituent of guar gum is galactomannan, a polysaccharide composed of galactose and mannose, synthesized in the seed endosperm through the galactomannan biosynthetic pathway. Despite its economic significance, little information is available on the structural and functional aspects of enzymes involved in this pathway. In the present study, key enzymes including α -D-galactoside galactohydrolase, β -1,4-mannan synthase (MS), phosphomannose isomerase (PMI), UDP-galactose 4-epimerase (UDP-G4E), and galactomannan galactosyltransferase (GGT) were characterized using bioinformatic approaches. Secondary and three-dimensional structures were predicted along with ligand-binding and active site identification. Physicochemical properties such as isoelectric point (pI), aliphatic index (AI), grand average of hydropathicity (GRAVY), instability index, hydrophobicity, and transmembrane activity were also analyzed, providing valuable insights into enzyme stability and functionality.

To complement the computational analysis, guar plants were subjected to drought stress to examine physiological and gum-related responses. Relative water content, photosynthetic rate, and transpiration rate significantly decreased under water deficit, whereas anthocyanin content increased, suggesting enhanced antioxidant defense against reactive oxygen species (ROS). Pod yield was reduced under stress; however, seed gum yield and viscosity increased. Furthermore, expression of genes involved in galactomannan biosynthesis was upregulated under drought conditions, indicating a stress-induced shift towards enhanced gum accumulation.

Overall, this multidisciplinary study integrates computational enzyme characterization with physiological and biochemical analyses, providing a comprehensive understanding of galactomannan biosynthesis and drought-induced gum modulation in *C. tetragonoloba*. These findings may facilitate targeted crop improvement strategies and industrial applications of guar gum under climate-stress scenarios.

Introduction

Cyamopsis tetragonoloba (guar or cluster bean) is a member of the Leguminosae family and has been valued since ancient times for its medicinal properties in treating microbial infections, tumors, inflammation, night blindness, and respiratory ailments (Wang and Morris 2007; Kumar et al. 2024). In addition to its medicinal value, guar is of high economic importance due to its seed endosperm, which is a rich source of gum with wide industrial applications. Guar gum has attracted considerable research attention for its thickening, stabilizing, and gelling properties in aqueous media, making it useful in food, pharmaceutical, and industrial formulations (Mudgil et al. 2014). The main constituent of guar gum is galactomannan, a polysaccharide consisting of a linear (1 $\rightarrow$ 4)- β -linked mannose backbone with (1 $\rightarrow$ 6)-linked α -D-galactopyranosyl side chains (Heyne and Whistler 1948). The mannose-to-galactose (M:G) ratio varies among species, with guar galactomannan typically having an M:G ratio of $\sim$ 1.6 (Edwards et al. 1992; Dos Santos et al. 2015). While pure mannan is insoluble in water, increasing galactose substitution enhances solubility by extending the polymer chain (Stephen 1983; Noble et al. 1986). Functionally, galactomannans contribute to water imbibition, drought avoidance during germination, and serve as carbohydrate reserves for seedling development (Grant Reid and Derek Bewley 1979). In guar, galactomannan is synthesized during seed development and stored as a mucilaginous layer in the endosperm (Kučka et al. 2022), accounting for 26–32% of seed dry weight (Kays et al. 2006).

Galactomannan biosynthesis is closely linked to central carbohydrate metabolism, with sucrose acting as a key connecting metabolite (Fig. 1). In plants, sucrose is transported from source leaves to sink tissues and is cleaved either by invertase into glucose and fructose or by sucrose synthase into UDP-glucose and fructose (Lammens et al. 2009). Glucose and fructose are phosphorylated by hexokinase to generate glucose-6-phosphate and fructose-6-phosphate, respectively. The latter is converted into mannose-6-phosphate (Man-6-P) by phosphomannose isomerase, which also functions in the reverse direction during mannose utilization (Sharma et al. 2014). GDP-mannose pyrophosphorylase and UDP-galactose 4-epimerase generate the activated sugar precursors GDP-D-mannose and UDP-D-galactose, respectively. Two tightly membrane-bound glycosyltransferases then catalyze polymer formation: GDP-mannose-dependent mannosyltransferase extends the β -1,4-linked mannose backbone, while UDP-galactose-dependent galactosyltransferase adds galactose residues via α -1,6 linkages (Sharma et al. 2022). The activities of these enzymes increase in parallel, maintaining a constant M:G ratio in the polymer. During germination, galactomannan degradation is carried out by β -mannosidase, β -mannanase, and α -galactosidase, which hydrolyze the mannan backbone, side chains, and oligomannans, respectively (Grant Reid and Meier 1973).

Although aspects of galactomannan yield have been studied in several species such as coffee (Pré et al. 2008), guar (Gresta et al. 2017), fenugreek (Wang et al. 2012), and *Caesalpinia pulcherrima* (Cerqueira et al. 2009), information on the genes and proteins of the galactomannan biosynthetic pathway remains limited. Reports are available on cDNA libraries for developing seeds of guar (Gresta et al. 2017) and coffee (Pré et al. 2008), but detailed structural and functional characterization of the pathway enzymes in guar is still scarce. Previous computational studies have successfully predicted enzyme structures and functions in secondary metabolic pathways, such as monoterpene biosynthesis in *Mentha* (Sanchita et al. 2014) and *Ocimum basilicum* (Yilmaz and Karik 2022), and triterpene saponin biosynthesis in *Centella asiatica* (Kumar et al. 2013).

In guar, the galactomannan biosynthesis pathway, gum content, and expression of biosynthetic proteins are also influenced by environmental stresses such as drought. Drought stress reduces physiological parameters including relative water content, osmotic potential, photosynthetic and transpiration rates, and pigment concentrations, while enhancing antioxidant accumulation (Pandey et al. 2022). Interestingly, pod yield decreases under stress, but gum yield per seed often increases, accompanied by altered expression of biosynthetic genes.

The present study therefore integrates computational and physiological approaches to analyze galactomannan metabolism in *C. tetragonoloba*. Specifically, we focus on structural and functional prediction of key biosynthetic enzymes, their binding site analysis, and the expression of pathway genes under drought stress. In parallel, we examine the physiological performance of guar under water deficit. Together, this information may help to unravel the regulation of galactomannan biosynthesis and provide a basis for improving gum yield and quality under stress conditions.

Materials and methods

Sequence retrieval

Sequence retrieval

The sequences of five proteins of galactomannan biosynthetic pathway of *Cyamopsis tetragonoloba* (Accession no. P14749, ADF47159, AAX31279, CAA32772, O65781, CAI79402) were retrieved from the protein sequence repository of NCBI database (www.ncbi.nlm.nih.gov/) (Table 1). The protein sequences were retrieved in FASTA format and used for further analysis.

Table 1
Protein sequences considered for the study

S. No.	Protein name	Accession no.	Length (AA)
1	α-D-Galactoside galactohydrolase	P14749.1	411
2	β-1,4-Mannan Synthase	ADF47159.1	526
3	Phosphomannose isomerase	AAX31279.1	428
4	UDP-Galactose 4-epimerase	O65781.1	350
5	Galactomannan Galactosyltransferase	CAI79402.1	435

Functional analysis

The function of a protein depends on its combination of domains (Fong and Marchler-Bauer 2008). For functional characterization of protein the conserved domains were searched through Conserved domain database (CDD) (Marchler-Bauer et al. 2011). The motifs were predicted using MEME suite (<http://meme.nbcr.net/meme/cgi-bin/meme.cgi>). The parameter of motif size was set at 6 and 50 as minimum and maximum, respectively. The gene ontology classification was also done by using Uniprot database (<http://www.uniprot.org>). Each protein was classified in three classes, biological process, molecular function and cellular component.

Primary Structure Analysis

Primary structure analysis or physico-chemical characterization was done by using the Expasy’s ProtParam server (<https://web.expasy.org/protparam/>). ProtParam was used to compute the biophysical and biochemical properties like isoelectric point (pI), molecular weight, instability index (Guruprasad et al. 1990), aliphatic index (Ikai 1980) and grand average hydropathy (GRAVY) (Kyte and Doolittle 1982).

Secondary Structure Prediction

CFSSP (<https://www.biogem.org/tool/chou-fasman/>) was employed for calculating the secondary structural features present in the protein sequences of galactomannan biosynthetic pathway considered in this study. The protein sequences were analyzed to predict the contribution of alpha helix, beta sheet and random coil structures (Chou and Fasman 1974) [30].

Model building and evaluation

The three dimensional structure of the protein was predicted by threading alignment modelling program using I-TASSER server (<https://zhanggroup.org/I-TASSER/>) (Yang et al. 2015; Zheng et al. 2021). The templates were selected from a link connecting to PDB database (<https://www.rcsb.org/>) consisting of known structures. The high resolution crystal structures of template proteins were considered for threading modelling. The template with maximum identity value and minimum e-score was selected for each protein sequence to build a model. The energy minimization was performed to stabilize the modelled protein with Swiss PDB Viewer (<https://spdbv.unil.ch/>) (Johansson et al. 2012). The model having higher C value (Confidence score) were chosen for further analysis. Structure visualization was performed through Pymol (<https://pymol.org/2/>).

Structure validation

After the model generation, its quality assessment was done, based on both geometric and energetic aspects. The stereochemical properties of obtained protein model were checked using RAMPAGE server (Lovell et al. 2003). The stereochemical properties of the proteins were assessed by Ramachandran plot analysis (Ramakrishnan and Ramachandran 1965). The validation for modelled structures was done to determine whether the residues were falling in the most favoured region in the Ramachandran plot by using PROCHECK (Laskowski et al. 1996).

Protein ligand binding site analysis

All modelled proteins were analyzed for ligand binding site by using COACH server (<https://zhanggroup.org/COACH/>). This server generates complementary ligand binding sites using two comparative methods, TM-SITE and S-SITE, which recognizes ligand binding templates from BioLiP protein function database

by binding specific substructure and sequence profile comparisons. These predictions will be combined with results from other methods including COFACTOR (<https://zhanggroup.org/COFACTOR/>), FINDSITE (Brylinski and Skolnick 2008) and ConCavity (<https://compbio.cs.princeton.edu/concavity/>) to generate final ligand binding site predictions.

Active site prediction

Protein-protein superposition, brings ligand into the target active site with an orientation similar to the one found in the original ligand-receptor crystal structure (Srivastava et al. 2010). The ligand binding site of modelled protein was predicted by superimposition of known template proteins by using COACH server (<https://zhanggroup.org/COACH/>). The RMSD was calculated using the superimposition between matched pairs. Ligand showing higher RMSD value and Z-value more than 1 were used for docking study. Active site residues and ligand binding site residues are shown in Table 9.

Table 9

Parameters used for the 3D structure, ligand binding site and active site prediction of the p

	Template used for modeling	Ident 1	Ident 2	Cov	Norm Z score	Cluster Size	C-Score	Lig Name	Lig binding Residues
1	1uasA	0.72	0.63	0.88	2.73	77	0.66	GLA	63, 98, 99,140, 148, 175, 177, 209, 211, 228, 232, 264
2	4hg6A	0.18	0.27	0.96	1.86	37	0.36	UDP	98,99,100,102,129,165,166,169,189,190,322
3	1pmiA	0.39	0.42	0.96	2.84	11	0.17	KOJ	55,108,117,119,144,146,282,284
4	4ouaB	0.09	0.20	0.93	1.47	5	0.14	OTR	38,177,178,181,193,194,195,196,199
5	3enkA	0.56	0.54	0.97	3.85	556	0.98	NAD	10,12,13,14,15,34,35,36,37,38,39,63,64,65,86,87,88,90,105,128,129,130,154,
1) α-D-Galactoside galactohydrolase 2) β-1,4-Mannan Synthase 3) Phosphomannose isomerise 4) Galactomannan Galactosyltransferase 5) UDP-Galactose									

Molecular docking

The docking of ligand with modelled protein was performed by Swiss Dock (<http://www.swissdock.ch/>) at ExPASy server. In this approach, the full flexible ligand was used for docking while keeping the protein in a fixed orientation in space. The negative and lower value of binding energy as well as more numbers of cluster involved showed favoured binding between ligand and target.

Plant material and drought experiment

Cluster bean (*Cyamopsis tetragonoloba*) seeds were grown in pots in soil and placed in growth chamber (PGC-105 HID; Percival Scientific, Inc., 505 Research Drive, Perry, IA 5022D, USA) for the study. The temperature variation was between 25–32°C and relative humidity was in the range of 50–70%. The photosynthetic photon flux density increased gradually from 6:00 to 12:00 h which ranged from (50–1300 μmol m⁻² s⁻¹) and then decreased till 7:00 pm to maintain light and dark cycle for the plants. The two months old plants were subjected to drought by withdrawal of water.

Measurement of relative water content

To measure relative water content of the varieties three to five mature leaves at 0, 1st, 5th, 10th day of drought and at rewatered stage were harvested early morning to measure the fresh weight. These leaf samples were kept overnight in water to measure the turgid weight (TW) the next day after they had attain turgidity and then these were kept in oven at 70°C for 72h. The dry weight was recorded and the relative water content RWC was calculated as

$$RWC (\%) = [(FW - DW) / (TW - DW)] \times 100.$$

Measurement of the osmotic potential

Osmotic potential of plants under different days of drought were determined in the leaves using a Wescor 5520 osmometer (Wescor, Logan, Utah, USA) as described by Ball and Oosterhuis (2005). Osmotic potential measurements were taken with leaf discs weighing approx 10-15mg each.

Gas exchange measurements

The net photosynthesis rate (*A*), stomatal conductance (*gs*), transpiration (*E*), and WUE were measured in fully expanded leaves of guar at the regular interval with portable photosynthesis system GFS-300 (Gas exchange and fluorescence measuring system). The photosynthetic photon flux density was maintained at 800 μmol m⁻² s⁻¹, CO₂ level in the leaf cuvette was 400 ppm, vapour pressure deficit (VPD) level was less than 2KPa and temperature of the leaf was at 30°C.

Chlorophyll estimation

The photosynthetic pigments such as chlorophyll and carotenoid were measured using method of Wellburn(Wellburn 1994). Leaf tissue of 20–25 mg was taken each from control and drought treated plants. These were kept in 1 ml of 80% acetone in dark for pigment extraction, then OD were recorded at 470, 647 and 663 nm for chl a, chl b and carotenoids determination.

Physicochemical characterization of gum

A Physiochemical property of the seed was measured as follows:

Sample collection

Seeds were collected from pods of guar plants of both watered and drought treated conditions. Seeds were soaked in water to remove endosperm and were kept in a refrigerator at 2–4° C for at most 24 h prior to isolation and purification.

Extraction and Isolation of the seed gum

Extraction and isolation of gum from seeds of guar were done using the procedure of Liyanage et al 2015 (Liyanage et al. 2015). The seeds were soaked in water to remove the endosperm for gum extraction. These were then dried in oven to remove moisture .Approximately (10–12 g) of the endosperm was grinded to a powder and left overnight in 50ml hexane. The powder was properly washed the next day in hexane and was filtered. The filtrate obtained was dried to remove excess hexane. 100ml water was then added to this and kept overnight. The next day it was filtered and washed in water for 2–3 times. The filtrate was centrifuged at 12000rpm for 10 mins and supernatant was taken. To this supernatant add ethanol (1.5 times the supernatant). A thick substance precipitates out as gum. This precipitate was oven dried at 50°C till it was powder. To purify the gum, the dried gum was dissolved in isopropanol (same volume as that of gum) with continuous stirring until completely dissolved and then finally dried.

Determination of Specific Rotation of Gums

Each gum sample (.01%) were filtered and equilibrated at 27° C of temperature. Optical rotations were determined for the three samples using polarimeter according to Dawber et al. (Dawber et al. 1988). The specific rotation of gum samples were measured in degrees.

Viscosity

Gum solutions of 0.01% were prepared by continuous stirring using a magnetic stirrer until the solution gelatinized. The actual viscosity of the gum was measured at 30° C at 6 rpm by a Well Brookfield Cone/Plate Microviscometer model R VT according to Stoloff et al (Stoloff 1958). The viscometer reading in centipoise (cP) was multiplied by a factor equivalent to the spindle number that has been used.

Ash and carbon content

Percentage ash and carbon content was determined using Analyser Multi N/C (Analytic Jena, Germany) 2100S. 40–50 mg of gum sample was used for estimation of carbon and ash content.

Results and discussion

Functional analysis of pathway proteins

Domains are evolutionarily conserved units in proteins and frequently correspond to recurrent structural and functional units (Basu et al. 2008). The particular function of a protein depends on its combination of domains; 80% of eukaryotic proteins have more than one domain. The galactomannan biosynthesis pathway proteins were analyzed for the availability of conserved domains. The domain having least e-value was selected corresponding to each protein. The retrieved conserved domains and their position in proteins were analyzed. Galactomannan Galactosyltransferase has single domains indicating that they did not participate in the formation of multi-domain structure. However α-D-Galactoside galactohydrolase, β-1,4-Mannan Synthase, Phosphomannose isomerase, UDP-Galactose 4-epimerase has two or three domains and participated in multiple domain structure (Table 2).Three motifs for each conserved domains were predicted. The motifs might be involved in binding site formation (Sanchita et al. 2013). The substrates of these proteins bind at these motifs of proteins (e.g enzymes). The motifs are represented in form of regular expression and web logo that explains the frequency of occurrence of residues of any motif at minimum 2 sites (Table 3).

Table 2
Conserved Domain found in pathway proteins

Protein	Conserved Domain	Position in the protein	E-value
α-D-Galactoside galactohydrolase	GH27	56–319	2.67e ⁻¹⁵⁹
	PLN02692	1-411	0e ⁺⁰⁰
β-1,4-Mannan Synthase	CESA_CaSu_A2	91–327	1.4e ⁻¹³⁷
	Glyco_tranf_2_3	91–327	1.51e ⁻²⁵
Phosphomannose isomerase	PLN02288	14–407	0e ⁺⁰⁰
	Man A	12–404	5.55e ⁻⁵⁵
UDP-Galactose 4-epimerase	UDP_G4E_1_SDR_e	5-334	0e ⁺⁰⁰
	PLN02240	1-349	0e ⁺⁰⁰
Galactomannan Galactosyltransferase	PLN03181	1-435	0e ⁺⁰⁰

Table 3
Domains with corresponding motifs and their regular expression

Protein	No. of Motifs	Regular expression	Logo	E-value	Width	Site count	R e entropy
α-D-Galactoside galactohydrolase	3	[RQ][YT][PP][MG]		3.3e ⁺ ₀₀₀	6	2	25.6
		[WR][SN][HF][SG][IC]		9.8e ⁺ ₀₀₀	6	2	24.2
		[KF][YF][SD][NM][CE]		1.2e ⁺ ₀₀₁	6	2	24.6
β-1,4-Mannan Synthase	3	A[IT][YKF]D[AL][LGD][FG][WQG][PKH]D[RHA] [TFD][FT]		4.1e ⁺ ₀₀₀	3	13	44.1
		Q[HA]RW[SK][FC]		1.3e ⁺ ₀₀₁	2	6	22.9
		[SH][LS[TRID]Y[ASH][FW]		5.3e ⁺ ₀₀₁	4	6	20.4
Phosphomannose isomerase	3	CS[VM][LK][TN]Y[KD][WQ]G		1.5e ⁺ ₀₀₀	2	9	36.3
		E[IC][ID][RE]C[MI]		3.8e ⁺ ₀₀₀	2	6	25.6
		[SI]Q[FA][HPD]		3.7e ⁺ ₀₀₁	2	6	23.9
UDP-Galactose 4-epimerase	3	I[GED]E[MID][CP]R[DG][IQ][YWP][NR][WNA]		8.4e ⁻ ₀₀₂	3	11	39.6
		E[QL][NE][WK][IA]		6.7e ⁺ ₀₀₁	2	6	23.8
		[QP][QL]V[MA][VA][GRRP]		8.1e ⁺ ₀₀₁	2	9	34.2
Galactomannan Galactosyltransferase	3	I[TL][LH][VF]TG[SC][QP][KC][RN][GC]H		7.2e ⁻ ₀₀₂	2	13	51.9
		D[VC]W[VN][SG]M		1.6e ⁺ ₀₀₁	2	6	24.6
		H[SG]W[TE][GL]		6.0e ⁺ ₀₀₁	2	6	23.8

Gene ontology classification of pathway proteins

The gene ontological classification of the proteins was analyzed in terms of biological process, molecular function and cellular component (Table 4). α-D-Galactoside galactohydrolase, Phosphomannose isomerase, UDP-Galactose 4-epimerase, enzymes involved in the carbohydrate metabolism while β-1,4-Mannan Synthase, Galactomannan Galactosyltransferase are the membrane bound enzymes and participated in cell wall organisation, biogenesis and degradation process. Phosphomannose isomerase, mannan synthase participated in mannan biosynthesis but PMI also involved in Zn ion binding. UDP-Galactose 4-epimerase catalyzes the formation of UDP galactose from UDP-glucose or fructose.

Table 4

Gene ontology classification of enzymes is dividing into three group biological process, molecular function and cellular component.

Protein	Biological Process	Molecular Function	Cellular Component
1 α -D-Galactoside galactohydrolase	Carbohydrate metabolic process, catalytic and enzymatic activity, α galactosidase activity and raffinose α galactosidase activity	Glycosidase, Hydrolase	
2 β -1,4-Mannan Synthase	Cell wall biogenesis and degradation, metabolic process and cell wall organization, GDP-mannose + (mannan)(n) = GDP + (mannan)(n + 1).	Transferase activity, transferring glycosyl groups	Golgi apparatus, and integral component of membrane
3 Phosphomannose isomerase	Carbohydrate metabolic process, GDP mannose biosynthetic process	Mannose-6-phosphate isomerase activity, zinc ion binding	
5 UDP-Galactose 4-epimerase	Carbohydrate metabolic process, galactose metabolic process, Ligand NAD	Coenzyme binding, UDP-glucose 4-epimerase activity, Isomerase activity	
6 Galactomannan Galactosyltransferase	Metabolic process, cell wall organisation	Transferase activity, transferring hexosyl groups	Golgi apparatus, and integral component of membrane

Physicochemical properties of proteins

Parameters computed using ExPASy's ProtParam tool given in Table 5. The calculated isoelectric point (pI) is useful because at pI, solubility is least and mobility in an electro focusing system is zero. Isoelectric point (pI) is the pH at which the surface of protein is covered with charge but net charge of protein is zero. At pI proteins are stable and compact. The computed pI value of all protein except α -D-Galactoside galactohydrolase is more than 7 indicating the net electrical charge of proteins is zero at basic pH value. However, α -D-Galactoside galactohydrolase showed less than 7 (pI < 7) reveals that net electrical charge is zero at acidic pH value. The computed isoelectric point (pI) will also be useful for developing buffer system for purification by isoelectric focusing method. The instability index provides an estimate of the stability of protein in a test tube. This method assigns a weight value of instability. Using these weight values, it is possible to compute an instability index. A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable (Guruprasad et al. 1990). The instability index value for these proteins was found to be ranging from 28.57 to 40.36. β -1, 4-Mannan Synthase and Phosphomannose isomerase showed comparatively high instability index 40. The very high aliphatic index of protein sequences indicates that β -1, 4-Mannan Synthase and Phosphomannose isomerase proteins may be stable for a wide temperature range. The lower thermal stability of α -D-Galactoside galactohydrolase, UDP-Galactose 4-epimerase and Galactomannan Galactosyltransferase was indicative of a more flexible structure when compared to other protein. The Grand Average Hydropathy (GRAVY) value for a protein is calculated as the sum of hydropathy values of all amino acids, divided by the number of residues in the sequence. GRAVY indices were found in the range from - 0.592 to 0.106. This low range of value indicates the possibility of better interaction with water. Negative value of hydropathy showed hydrophilic proteins while positive showed hydrophobic nature of the protein (Kyte and Doolittle 1982). Except β -1, 4-Mannan synthase all studied proteins showed negative value of hydropathy and these are hydrophilic nature towards water.

Table 5

Physicochemical parameter of pathway proteins is computed by using ExPASy's ProtParam tool.

Protein	AA	Mol wt	pI	Instability Index	Aliphatic Index	GRAVY
α -D-Galactoside galactohydrolase	411	45135.5	4.97	32.11	76.69	-0.359
β -1,4-Mannan Synthase	526	60677.4	9.28	40.35	100.78	0.106
Phosphomannose isomerase	428	47773.4	6.60	40.36	89.72	-0.223
UDP-Galactose 4-epimerase	350	38373.7	6.72	28.57	87.74	-0.198
Galactomannan Galactosyltransferase	435	50956.0	7.98	35.57	72.44	-0.592

Structural analysis of modelled pathway proteins

The secondary structure of proteins was analyzed for considering relative availability of alpha helix, extended strand and random coils. The percentage distribution of predicted secondary structure features are represented in Table 6. The results revealed that the predicted alpha helix dominated among secondary structure elements followed by extended strand, random coils for all protein sequences. Present study findings were similar to the earlier results reported in *Withania somnifera* (Sanchita et al. 2014).

Table 6
Secondary structure elements identified in proteins.

Protein	Alpha Helix (%)	Extended strand (%)	Random Coil (%)
α -D-Galactoside galactohydrolase	62.5	59.6	14.6
β -1,4-Mannan Synthase	85.7	53.8	9.5
Phosphomannose isomerase	82.2	67.3	13.1
UDP-Galactose 4-epimerase	68.0	38.6	12.9
Galactomannan Galactosyltransferase	62.5	61.1	13.1

Three-dimensional structures were predicted for proteins where such data is unavailable. There is lack of experimental structures for the proteins considered. The modelling of the three dimensional structure of these proteins were performed by threading alignment web server, I-TASSER. The template having maximum identity (maximum alignment coverage) and highest c value was selected and models were created using I-TASSER server. The model having lowest energy was considered for further study (Fig. 2). The stereochemical quality and accuracy of the predicted models were evaluated using Ramachandran map calculations computed with the PROCHECK program. The result revealed that the modelled structure for all protein sequences have 76.5%, 72.7%, 74.3%, 76.5%, 80.9% and 56.2% residues for α -D-Galactoside galactohydrolase, β -1,4-Mannan Synthase, Phosphomannose isomerase, UDP-Galactose 4-epimerase and Galactomannan Galactosyltransferase respectively in most favoured region (Table 7). The identified Ramachandran plots represent a good quality of predicted models. The structures were naturally stable with low energy value. The RMSD values identified through superimposition between selected template structure and modelled structure.

Table 7
Percentage distribution of amino acid residues in ramachandran plot.

Protein	Residues in most favored region (%)	Residues in additionally allowed region (%)	Residues in generously allowed region	Residues in disallowed region
α -D-Galactoside galactohydrolase	76.5	19.0	3.1	1.4
β -1,4-Mannan Synthase	72.7	19.5	4.6	3.1
Phosphomannose isomerase	74.3	21.7	2.1	1.9
UDP-Galactose 4-epimerase	80.9	15.7	3.0	0.3
Galactomannan Galactosyltransferase	56.2	32.8	7.3	3.6

Ligand binding site prediction for modelled proteins

Modelled proteins of galactomannan biosynthetic pathway were analyzed for ligand binding site prediction. PMI showed minimum number of ligand binding residues while UDP-G4E has maximum. α -D-Galactoside, MS, PMI, GT, UDP-G4E have shown GLA, UDP, Zn, OTR and NAD as probable ligands, respectively (Fig. 3). All modelled proteins showed more than 88% sequence coverage of templates. GT have minimum clusters size while UDP-G4E showed maximum templates in a cluster (Table 8).

Table 8
Trans-membrane activity of pathway proteins

	No of transmembrane helices	Start from	End to	Helices length	Score	Orientation
α -D-Galactoside galactohydrolase	1	124	143	20	2255	o-i
β -1,4-Mannan Synthase	4	78	100	23	1901	o-i
		409	428	20	25554	i-o
		438	467	30	771	o-i
		548	571	20	2160	i-o
Phosphomannose isomerase	2	303	322	20	832	o-i
		413	432	20	630	i-o
UDP-Galactose 4-epimerase	2	50	69	20	516	i-o
		112	133	22	752	o-i
Galactomannan Galactosyltransferase	2	23	43	21	527	i-o
		77	98	22	2531	i-o

Active site prediction in the modelled proteins

All modelled proteins were also analyzed for active sites (Fig. 4). MS, PMI and GT did not show any active site residue while α -D-Galactoside and UDP-G4E have 2 and 5 active site residues, respectively (Table 8). MS, PMI and GT also showed less c value for the active site prediction. These are membrane proteins and for membrane proteins c value is always less. $C_{\text{Score}}^{\text{EC}}$ is the confidence score for the EC number prediction. $C_{\text{Score}}^{\text{EC}}$ values range between 0–1, where a higher score indicates a more reliable EC number prediction. TM score is a measure of global structure similarity between query and template protein. RMSD^a is the RMSD between residues that are structurally aligned by TM align. IDEN^a is the percentage sequence identity in the structurally aligned region.

Docking study of pathway protein with their ligands

Molecular docking can fit molecules together with a favorable configuration to form a complex system. The structural information from the theoretical modeled complex can help us to understand the catalytic mechanism of an enzyme. The modelled pathway proteins were studied for docking with their probable ligand respectively. UDP-G4E showed minimum enthalpy energy and fitness energy thus it forms more stable UDP galactose which further converted in to galactomannans (Table 9). However, PMI showed comparatively higher enthalpy change and catalyze reversible reaction and forms less stable compound. Ligand binding at appropriate configuration (with lowest enthalpy change) is given in Fig. 5.

Physiological performance of the guar plants under drought stress

Milan variety of guar was imposed to drought for 10 days. Relative water content of the leaf dropped up to 50% at the 10th day of drought (DD) (Fig. 6A). Osmotic potent also decreased under the drought and plants were not fully recovered after the one week of recovery (Fig. 7B). The photosynthesis rate (A) was decreased by 66.2% in 10DD, however stomatal conductance (g_s) and transpiration rate (E) did not change significantly under drought stress (Fig. 7A, B, C). Water use efficiency (WUE) decreased by 61% under drought stress. However, fully recovered in one of recovery (Fig. 7D). The chlorophyll content decreased up to 60% in comparison to watered plants. Chlorophyll a/b ratio did not changed significantly under drought stress (Fig. 8A, B). carotenoid content increased dramatically at the 5th DD and did not recovered after the recovery period (Fig. 8C). Anthocyanin content increased upto 80 μmol per gram of fresh weight. Further it decreased after the rewatering of one week (Fig. 8D). Drought causes decrease in water content which is accompanied by loss of turgor and wilting, closure of stomata, reduction in photosynthesis, and interference with many other basic metabolic processes (Singh et al. 2014). Drought stress primarily decreases the photosynthesis rate due to decrease in internal CO_2 concentration resulting from stomatal closure (Chaves et al. 2009; Vishnyakova et al. 2023). Chlorophyll and carotenoid are light harvesting photosynthetic pigments which showed changes to avoid the inhibition in photosynthesis reaction centers under drought stress. These pigments also degrade which leads to deterioration of thylakoid membranes (Munné-Bosch and Alegre 2000). Carotenoids act as accessory light harvesting pigments thus transferring light energy to chlorophylls. Anthocyanins are another class of pigments which also have photoprotective effect. Under stress conditions to avoid photooxidative damage to plant tissues anthocyanins functions as antioxidants by scavenging reactive oxygen radicals (Ranjan et al. 2014). These act as light attenuators by not transferring the absorbed light energy to the photosynthetic machinery of the plants thus protecting underlying cells from high irradiance (Hogewoning et al. 2012; Zhu et al. 2016). Our study revealed that the anthocyanin content is found to increase under drought which is a protective mechanism of plant against stress.

Gene expression of the proteins involved in the galactomannan biosynthesis

Fru-6-P converts into Man-6-P by phosphomannose isomerase (PMI) which also functions in the reverse direction for the utilization of mannose. Expression of PMI is decreased by 15.8 fold in comparison to watered plants (Fig. 9). UDP-G4E is the enzyme which forms the direct precursor for galactomannan biosynthesis, UDP-D-galactose. In this study expression value was upregulated by 5.8 fold in comparison to control plants (Fig. 9). UDP-galactose-dependent GT transfers a galactose residue through a α -linkage to a mannose near the nonreducing end of the growing mannan chain. Expression of GT is upregulated by 20 fold while MS increased by 60.5 fold in comparison to watered plans (Fig. 9). Galactosidase is an enzyme which hydrolyse the galactose residue of galactomannan. Expression of galactosidase is down regulated by -30.6 fold in comparison to control plants (Fig. 9). Overall enzymes involved in the galactomannan biosynthesis are upregulated. However, enzymes associated with hydrolysis are downregulated. Formation of seed and fruit influenced by various metabolic processes occurred in leaves and translocated in to seeds. Drought stress affect the physiological and biochemical process which involved in the grain filling and grain quality (Sehgal et al. 2018; Boote et al. 2023). Although seed yield were decreased under water stress but seed gum yield increased under drought stress.

Effect of drought on gum yield and its physiochemical properties

The physiochemical properties of seed gum were studied. The yield of gum was increased with 1.9 fold under drought stress (Table 11). The specific rotation of .01% sample was decreased by 1.1 fold under drought stress. Drought stress increased relative conductivity of gum samples. Percentage of ash in gum is believed to be the measure of mineral quantities in the guar seed (Dakia et al. 2008; Zammel et al. 2021). The percentage of ash showed significant decrease by 2.2 fold under water stress. Measurements of viscosity showed increased by 1.2 fold (Table 11). The specific rotation of organic molecules such as carbohydrates is related to their structure and is a characteristic property of the substance (Sathyanarayana and Stevens 1987). The specific rotation of gum is thus considered as the most important criterion of purity and qualitative analysis. The percentage ash content indicates the presence of inorganic elements and mineral quantities in gum. The viscosity of any liquid represents its resistance to shearing and stirring. It is an important analytical parameter which depends on shape and size of the macromolecule (Anderson 1978). Viscosity is the most characteristic feature of guar gum it is due to its property to hydrate in aqueous solutions. Viscosity of the gum depends on several factors such as temperature, time, pH, concentration and ionic strength and type of agitation (Mudgil et al. 2014). It was observed that drought increased viscosity of the gum solutions. This property of viscosity is due to galactomannan which may increase during drought stress (Hoppe and Goswami 1999).

Table 11
Gum yield and rheological properties of seed gum

Parameter	Control	Drought
Gum Yield (%)	8.67 ± 1.53	15.87 ± 1.87
Optical Rotation ($\mu\text{S cm}^{-1}$)	60.51 ± 3.89	54.78 ± 4.23
Viscosity (cP)	25.89 ± 3.78	32.45 ± 2.67
Carbon content (%)	34.75 ± 2.35	39.56 ± 33.12

Conclusion

Cyamopsis tetragonoloba is an important legume crop used for medicine and seed gum. Seed gum is made up of galactomannan which is a polysaccharide. It is synthesized by a divert pathway from the central carbohydrate metabolism. Drought stress affects physiological and biochemical metabolism. It diverted carbon metabolism towards sink tissue. Relative water content, photosynthesis rate, transpiration rate, chlorophyll content decreased under water stress. However carotenoid and anthocyanin content increased during drought. These act as light attenuators by not transferring the absorbed light energy to the photosynthetic machinery of the plants thus protecting underlying cells from high irradiance. α -D-Galactoside galactohydrolase, β -1,4-Mannan Synthase (MS), Phosphomannose isomerase (PMI), UDP-Galactose 4-epimerase (UDP-G4E) and Galactomannan Galactosyltransferase (GGT) are the proteins involved in galactomannan biosynthesis. Therefore these proteins are studied in silico and gene expression analysis. Three-dimensional structures, ligand binding site, active sites were also predicted for these pathway enzymes. The physico-chemical properties such as pI, AI, GRAVY, instability index, hydrophobicity and transmembrane activity were also studied. The current information will provide a platform to know the structural and functional attributes of these proteins for further studies.

Table 10
Docking parameters for pathway proteins

Name of protein	Cluster	Element	Full fitness (Kcal/mol)	Estimated ΔG (Kcal/mol)
α -D-Galactoside galactohydrolase	37	0	-1231.78	-6.06
β -1,4-Mannan Synthase	32	0	-936.42	-10.25
Phosphomannose isomerase	38	0	-1689.90	-5.23
UDP-Galactose 4-epimerase	30	4	-1929.91	-11.17
Galactomannan Galactosyltransferase	8	2	-1212.81	-6.19

Declarations

Conflict of interest

The author declares that no conflicting interests exist.

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Author Contribution

RS designed and conceptualized the research, performed the identification, analysis and physiological study of the control and drought treated plants. She also performed docking between protein and substrate and critically revised manuscript. All authors read and approved the manuscript.

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Figures

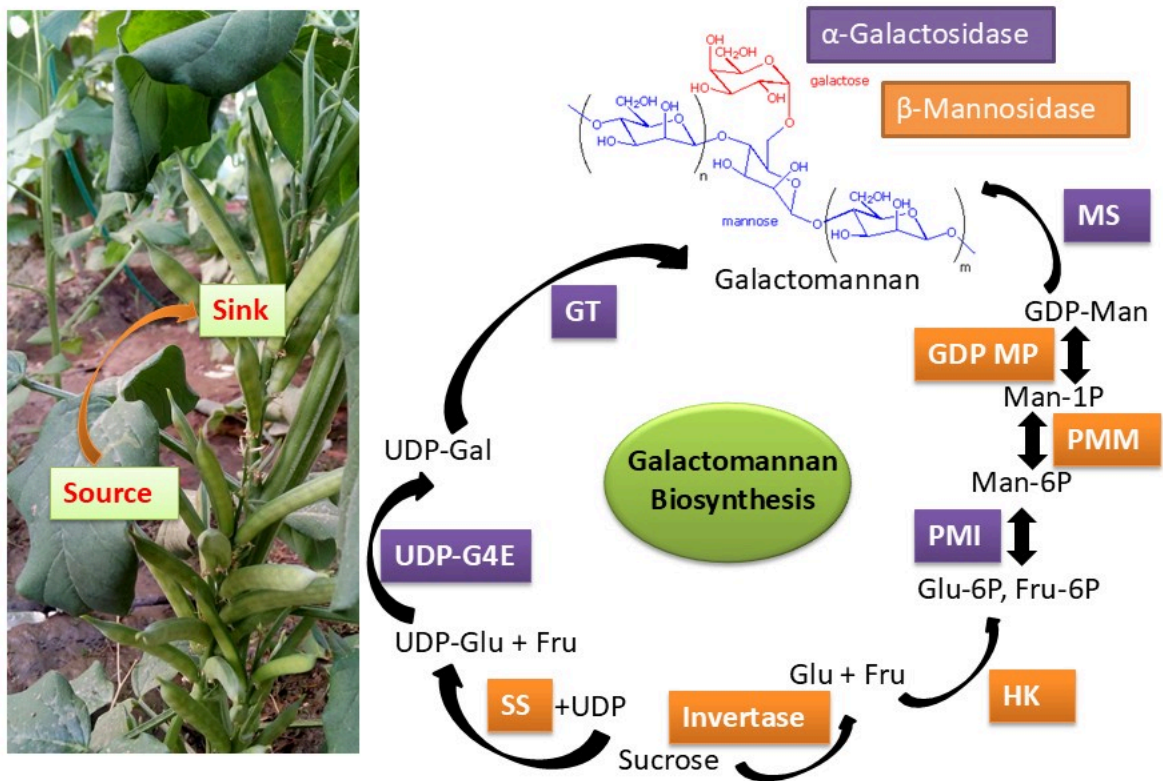


Figure 1

Figure 1

Schematic representation of galactomannan biosynthesis.

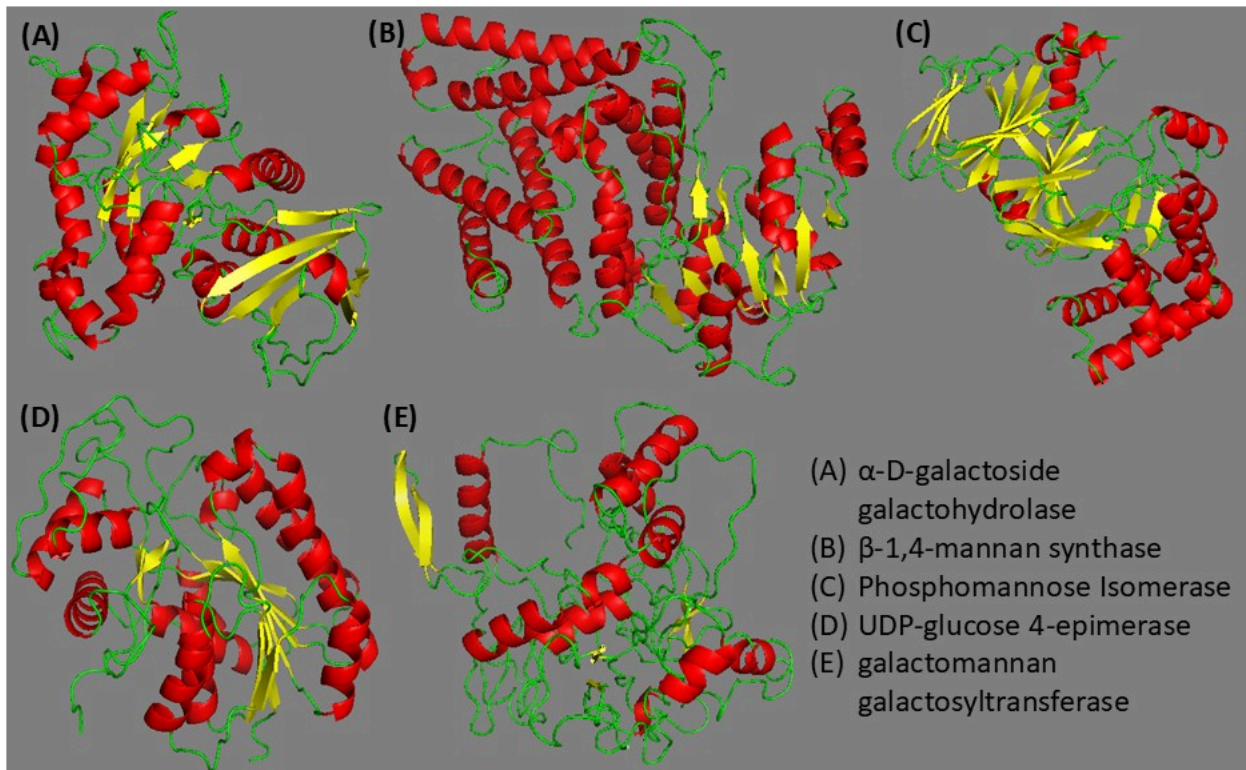


Figure 2

Figure 2

Three-dimensional structure of the modelled proteins of galactomannan biosynthesis

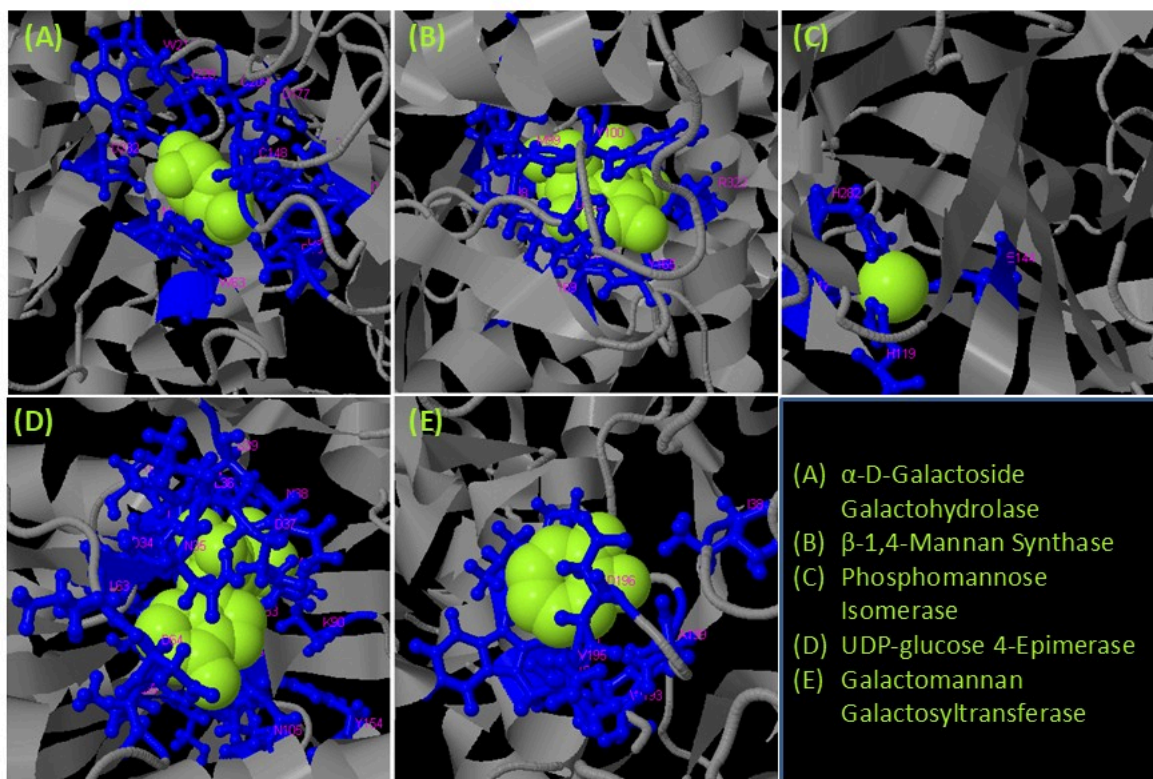


Figure 3

Figure 3

Predicted ligand binding sites in the modelled proteins of galactomannan biosynthesis

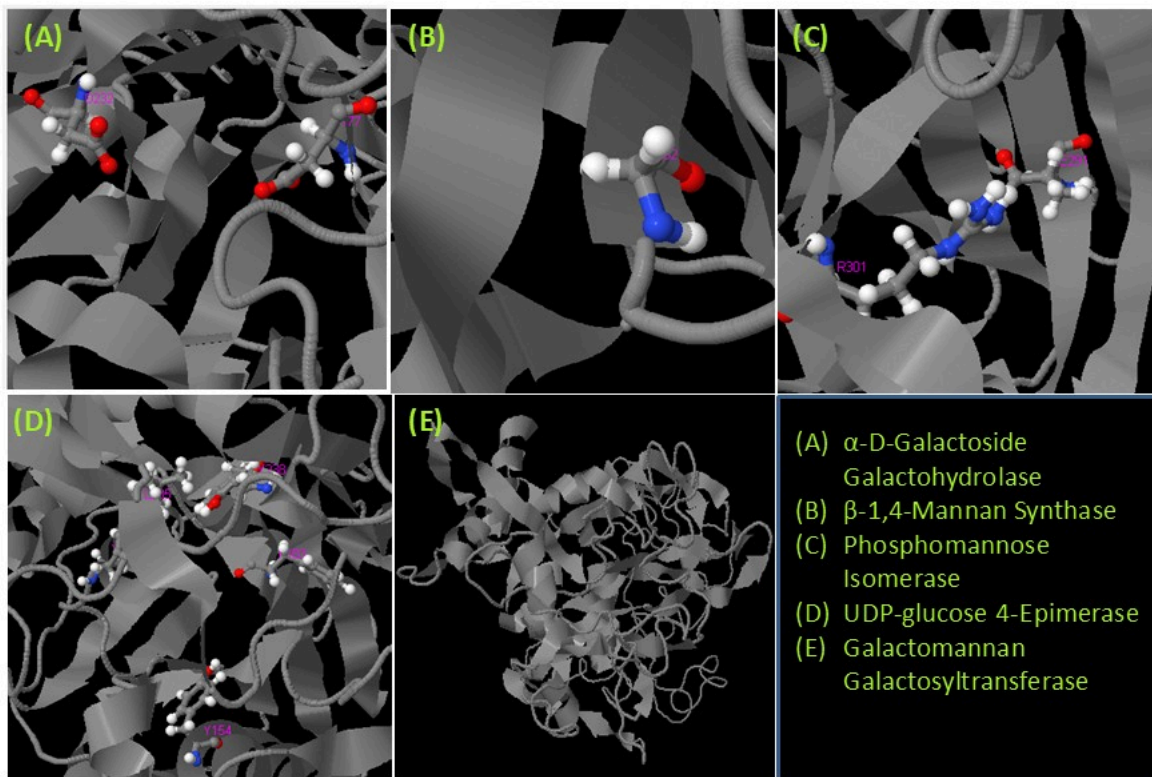


Figure 4

Figure 4

Predicted active sites in the modelled proteins of galactomannan biosynthesis

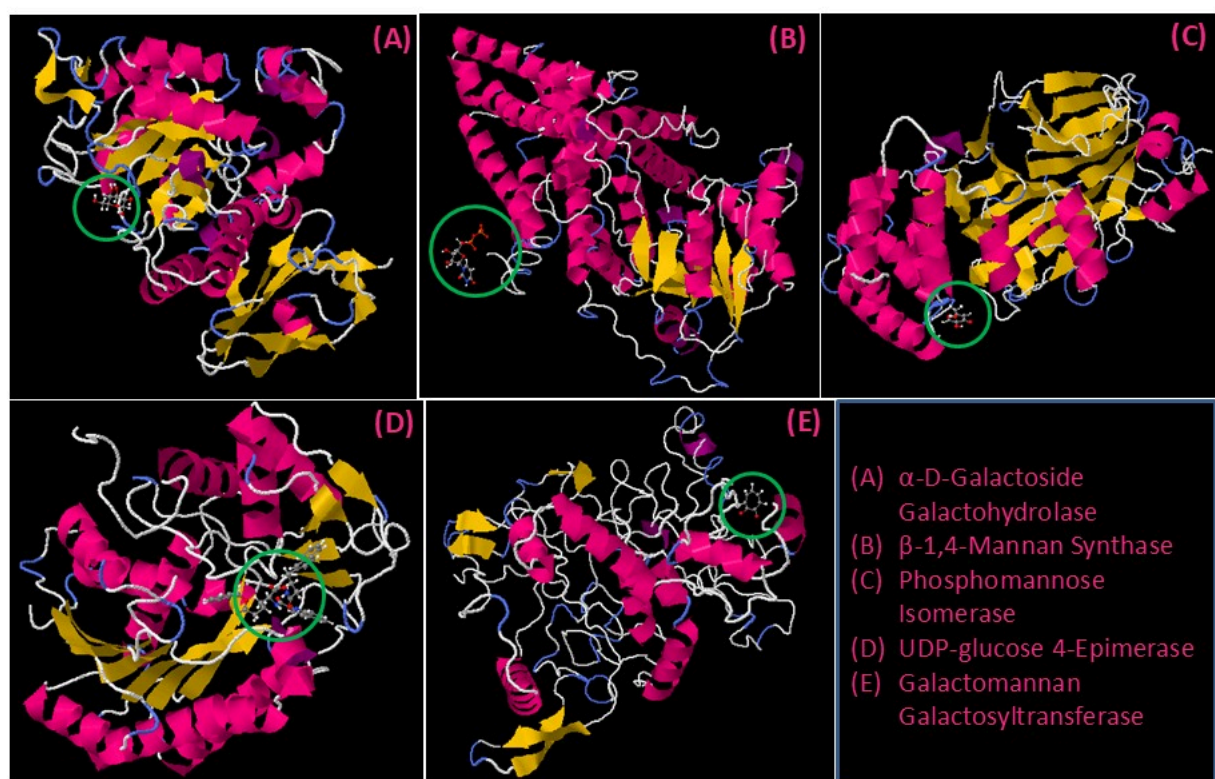


Figure 5

Figure 5

Docking of proteins of galactomannan biosynthesis with corresponding ligands

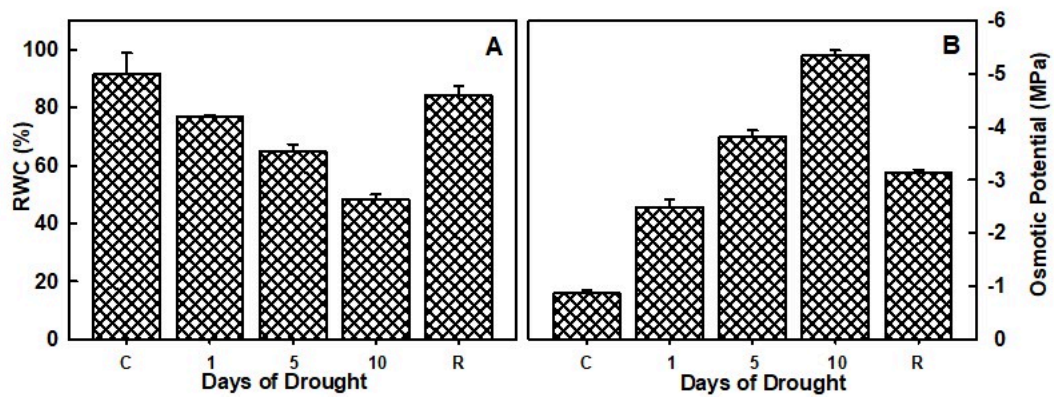


Figure 7

Figure 6

Relative water content, Day RWC (%) (A). Predawn leaf water potential (B) measured in the leaves of *Cyamopsis tetragonoloba* under irrigated and water-deficit conditions on the 1st, 5th, and 10th day of drought stress (B). Each bar represents $\pm$ SD of five measurements

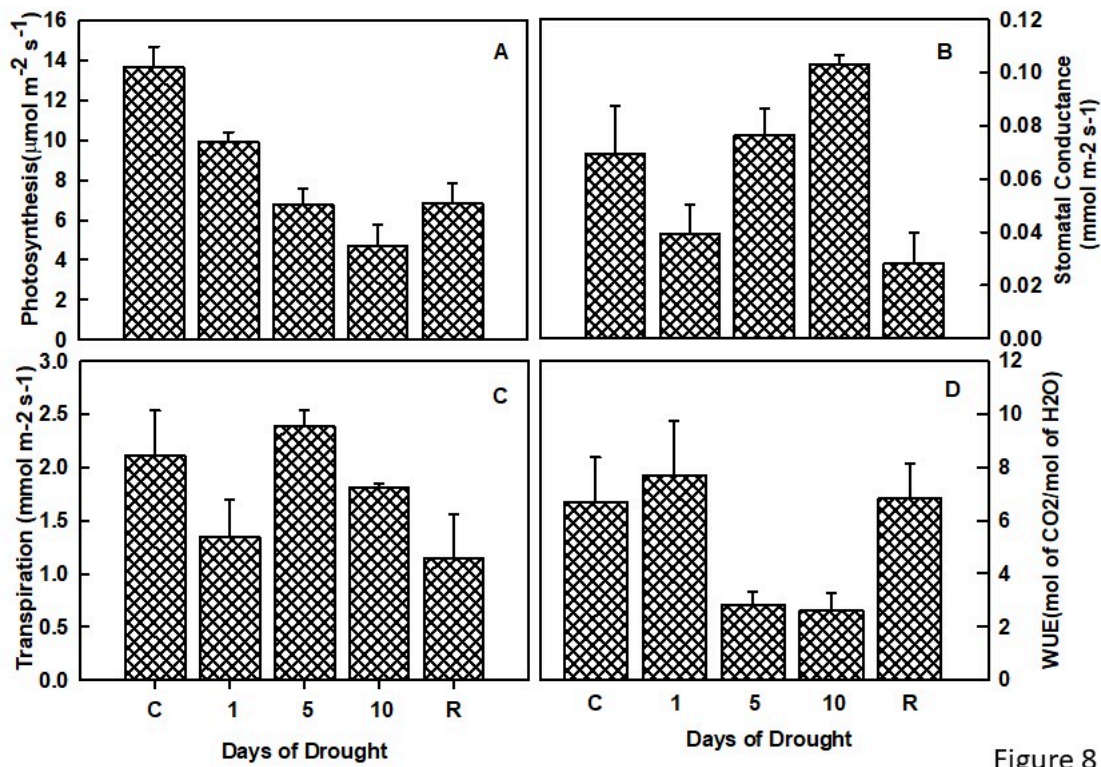


Figure 8

Figure 7

Effect of drought stress on net photosynthesis (A), stomatal conductance (B), transpiration rate (C), and water use efficiency (WUE) (D) in *Cyamopsis tetragonoloba* on the 1st, 5th and 10th day of drought stress. Data represent the means $\pm$ SD of five separate measurements.

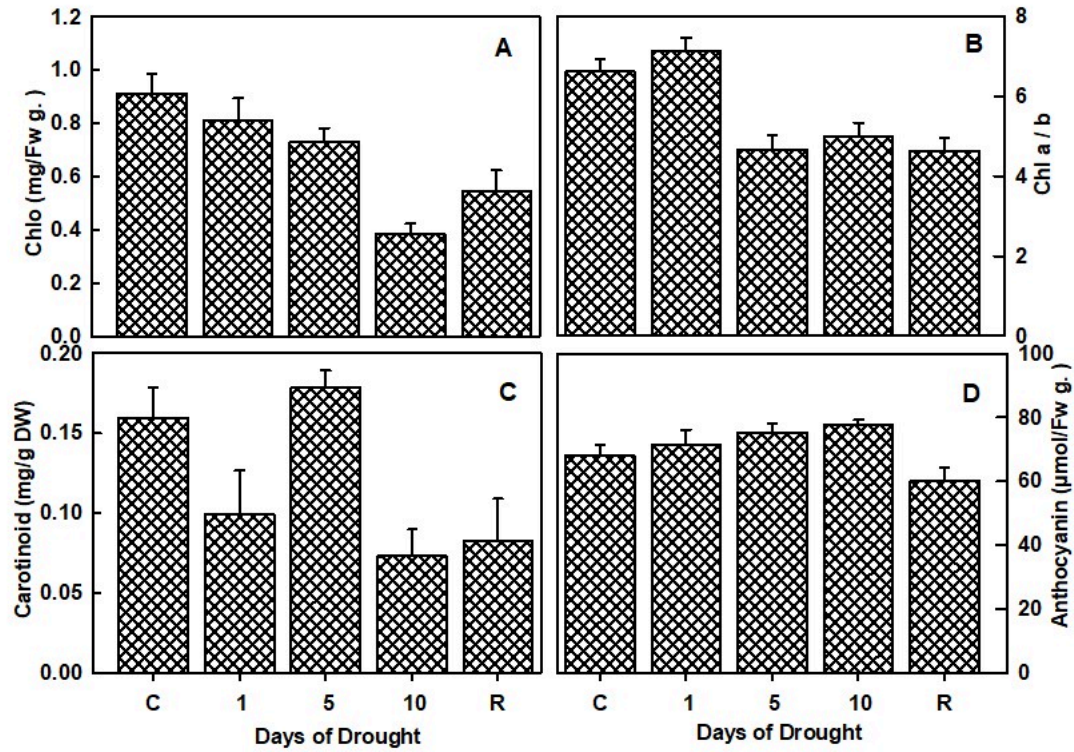


Figure 9

Figure 8

Chlorophyll a (A) chlorophyll a/b, carotenoid (C) and anthocyanin (D) content of *Cyamopsis tetragonoloba* on the 1st, 5th and 10th day of drought stress. Data represent the means $\pm$ SD of five separate measurements.

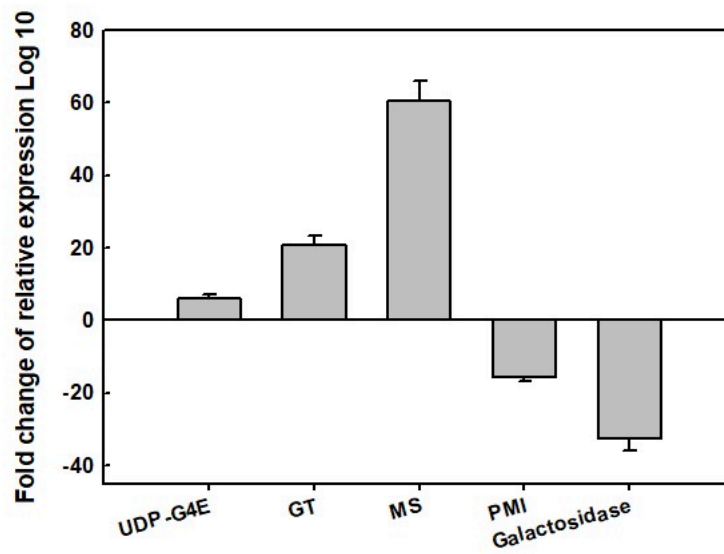


Figure 10

Figure 9

Response of gene expression under drought stress in α -D-Galactoside galactohydrolase, β -1,4-Mannan Synthase (MS), Phosphomannose isomerase (PMI), UDP-Galactose 4-epimerase (UDP-G4E) and Galactomannan Galactosyltransferase (GGT) under control and the 10th day of drought condition in *Cympopsis tetragonoloba*. Results are presented as the ratio of expression of each gene (relative to that of ubiquitin) in drought-treated plants compared to watered controls, transformed in log10. Error bars indicate the SD of the mean (n=3)