

Sequence Analysis and Homology Modeling of Seed Storage Proteins from Different Species of Beans

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Abstract: Beans provide essential proteins in human diet, complementing other food sources. Information on genetic diversity and relationships among crop species is essential for the efficient explanation of taxonomic relationships. Researchers can use information on genetic similarity to make decisions regarding selection of superior genotypes for improvement or for use as parents for the development of future cultivars. Seed storage proteins of beans associated with different functions are phaseolin, phytohemagglutinins, hydrolases like amylases, proteinases, lipases and cellulases, proteinase inhibitors, lectins, lectin-like proteins, ribosome-inactivating proteins, allergens, glucanases, chitinases etc. Information regarding the genetic similarity of beans seed proteins is limited. Hence, the study investigated the sequence analysis of seed storage proteins in *Phaseolus vulgaris*, *Vigna unguiculata* and *Cajanus cajan*. Protein sequences were obtained from NCBI database and multiple sequence alignment was performed with clustalX-2.1 while homology modeling and structural alignment was achieved with SWISS-MODEL and PyMol respectively. The result showed that β -amylase and other seed storage proteins in *Phaseolus vulgaris*, *Vigna unguiculata* and *Cajanus cajan* which are associated with pests and pathogens control are more conserved and structurally related than the other seed proteins. The high structural similarity, sequence conservation and identities of the studied proteins suggest that they have similar functions in the bean species. This could be exploited for development of pest and/or pathogen resistant bean species.

Key words: Beans • Seed storage proteins • Sequence conservation • Homology modeling • Sequence analysis

INTRODUCTION

Beans provide essential proteins in the human diet, complementing other food sources like maize and rice [1]. Legume and oilseed proteins entail about 15 to 50 % of the dietary proteins for humans in many countries. The common bean (*Phaseolus vulgaris* L) is one of the most consumed grain legumes in the world, mainly in South America and Africa [2]. Among our food sources, plants of the legume family contribute some of the most important protein-rich seeds [3]. Seed storage proteins have been classified into four families, termed 11S globulin (also known as α -conglutin, legumin, legumin-

like and glycinin), 7S globulin (also known as β -conglutin, vicilin, convicilin and vicilin-type), 7S basic globulin also known as g conglutin) and 2S sulphur-rich albumin also known as δ conglutin) [4]. Major seed storage protein of common bean are phaseolin (vicilin-like 7s globulin), phytohemagglutinins (PHA), some wild accessions contain third abundant protein called Arc. Seed storage proteins involved in mobilization of different compounds that are used during germination are hydrolases like amylases, proteinases, lipases and cellulases while those that act against pests and pathogens are proteinase inhibitors, lectins, lectin-like proteins (arcelins [Arc] and α -amylase inhibitors [α AI]), ribosome-inactivating

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proteins, allergens, lipid transfer proteins, glucanases and chitinases [3]. For example, various species of the genus *Phaseolus* contain a family of related lectins and lectin-like proteins (LLP) that are associated with antibiosis activity against seed storage pests [5]. Also, a protease inhibitor from *Mucuna pruriens* has been reported to provide protection against the effects of snakebite [6] while phaseolin seed coat of *Pharsalus lunatus* deters larval development of in bruchid [7].

Computational tools and resources can help researchers to study physicochemical and structural properties of proteins including sequence similarity. A large number of computational tools and resources are available from different sources for making predictions regarding the identification and structural prediction of proteins. The major drawbacks of wet experimental methods that have been used to characterize the proteins of various organisms are the time frame involved, high cost and the fact that these methods are not amenable to high throughput techniques. *In silico* approaches provide a viable solution to these problems. The amino acid sequence provides most of the information required for determining and characterizing the molecule's function, physical and chemical properties. Computationally based characterization of features of proteins found or predicted in completely sequenced proteomes is an important task in the search for knowledge of protein function. Hence, the study investigated the sequence analysis of seed storage proteins in *Phaseolus vulgaris*, *Vigna unguiculata* with its varieties and *Cajanus cajan*.

MATERIALS AND METHODS

Sequence Retrieval: National Center for Biotechnology Information (NCBI) database (<https://www.ncbi.nlm.nih.gov/genbank/>) was screened with the words *Phaseolus vulgaris*, *Vigna unguiculata* (and its varieties) and *Cajanus cajan* and a total of ninety one (91) seed storage proteins with following accession numbers were identified and obtained in FASTA format. A subtotal of fifty seven (57) seed storage proteins (AAA33756, AAC23610, AAB23263, CAA36853, AAA99534, AAC04316, AAA67353, AAA67354, CAA85418, 2PHL_A, AAC78504, CAA40474, IIOA_A, 1FAT_A, CAA85405, CAA90585, BAA33879, 1AVB_A, AAF23725, CAD27954, CAD28673, CAD28675, CAD28677, CAD28836, CAD28838, CAD28839, CAD28840, CAD29132, CAD29133, CAD29134, CAD58657, CAD58972, CAE00464, ADR30064, ADR30065, ADR30068, ADR30069, ADR30070, ADR30071,

ADR30072, ADR30073, ADR30074, CCF55433, CCF55434, CAA26718, AAA67352, AAA67355, BAA05105, AAB50853, AAB50854, CAB17074, CAB17075, CAB17076, CAB17077, AAL23841, CAD28676, AAT35809) were identified for *P. vulgaris*, a subtotal of twenty nine (29) (CAA61280, CAA61279, CAA61278, CAA61281, CAA12395, AAD34914, CAC81081, CAC81820, AAO43979, AAO43980, AAQ14319, AAQ14346, ABD85194, ABQ32293, ABQ32297, ABU55377, CAP19902, AAA33140, AAA33141, AAA33143, AAA33142, AAO43981, CAM35517, CAM35518, CBJ34319, AAG23965, AAO43982, AAO43983, 4TX7_A) were identified for *V. unguiculata* while only five (AAK61346, AEW50184, AAV51976, ADB44827, AAP49847) were identified for *C. cajan*.

Sequence Alignment and Phylogenetic Tree

Construction: The above protein sequences were aligned with ClustalX-2.1 [8]. Briefly, default multiple alignment parameters of Gap opening [0 - 100] of 10, Gap extension of [0 - 10] of 0.2, Delay divergent sequence of 30 % and protein weight matrix of BLOSUM series deployed for the sequence analysis using UPGMA clustering algorithm. The aligned sequences were written as postscript file while the phylogenetic trees were generated in Phylip format and plotted with NJ-plot [9]. Sequence similarity and diversity were deduced by visual inspection of the clusters in the dendrogram. Protein sequences that are closely related between different bean species were identified and further subjected to analysis and comparative homology modeling.

Homology Modeling, Model Validation and Structural

Alignment: The closely related seed storage proteins in the different bean species were identified from the dendrogram. The comparative homology modeling of 3-D structures of the closely related proteins was achieved with SWISS-MODEL online tool [10, 11] using automatic mode. Template search with Blast and HHblits was performed against the SWISS-MODEL template library. The target sequence was searched with BLAST [12] against the primary amino acid sequence contained in the SWISS-MODEL Template Library (SMTL). An initial HHblits profile was built using the procedure outlined in Remmert *et al.* [13], followed by 1 iteration of HHblits against NR20 and the obtained profile was searched against all profiles of the SMTL and templates were selected. Template's quality was predicted from features of the target-template alignment and the templates with highest quality were selected for model building.

Models are built based on the target-template alignment using ProMod3 and Coordinates which are conserved between the target and the template are copied from the template to the model. Insertions and deletions are remodelled using a fragment library while side chains are rebuilt. Finally, the geometry of the resulting model is regularized by using a force field. Where loop modeling failed with ProMod3, an alternative model is built with PROMOD-II [14]. The global and per-residue model quality was assessed using the Qualitative Model Energy Analysis (QMEAN) scoring function [15]. Ligands present in the template structure were not transferred by homology to the model because the following criteria were not met (a) The ligands are annotated as biologically relevant in the template library, (b) the ligand is in contact with the model, (c) the ligand is not clashing with the protein, (d) the residues in contact with the ligand are conserved between the target and the template. Models were generated and ranked according to their sequence identity with their respective templates. The models were considered sufficiently reliable when there is more than 50 % sequence identity between the template and the targets proteins [10]. Structural alignment of the modeled beans proteins with their respective templates was aligned and visualized with PyMol-1.4.1.

RESULTS AND DISCUSSION

Sequence alignment of seed storage proteins from *P. vulgaris*, *V. unguiculata* and *C. cajan* shows that the studied bean species are related with respect to their seed storage proteins (Figure 1). Figure 1 shows that CAA61281 (Chitinase class 4 from *V. unguiculata*) and CAA40474 (Chitinase from *P. vulgaris*) are closely related. Also, ABD85194 (Trypsin inhibitor from *V. unguiculata*) and AAL23841 (Trypsin proteinase inhibitor from *P. vulgaris*) are closely related. It was also observed that CAM35518 (Lectin from *V. unguiculata ssp cylindrica*) and AEW50184 (Lectin from *C. cajan*) are closely related while CBJ34319 (Kunitz-type protease inhibitor from *V. unguiculata ssp cylindrica*) and ADB44827 (protease inhibitor from *C. cajan*) is closely related (Figure 1).

Specifically, chitinases and trypsin inhibitors of seeds of *P. vulgaris* and *V. unguiculata* are closely related while lectins, protease inhibitors in seeds of *V. unguiculata subsp cylindrica* and *C. cajan* are closely related. It could be seen from Figure 1 that *P. vulgaris* and *C. cajan* are quite divergent with respect to their seed storage proteins. The present study showed that chitinase with accession numbers of CAA40474 and

CAA61281 from *P. vulgaris* and *V. unguiculata* respectively are homologs of class IV chitinase from *Zea mays* (maize) (Table 1 and Figure 2). Also trypsin inhibitors with accession numbers of AAL23841 and ABD85194 from *P. vulgaris* and *V. unguiculata* respectively are homologs of Bowman-Birk serine protease inhibitor (Bowman-Birk type trypsin and chymotrypsin inhibitor) from *V. unguiculata* (Table 1 and Figure 2).

It was equally observed that lectins with accession number of AEW50184 from *C. cajan* is a homolog of lectin from *Lens culinaris* (lentil) while CAM35518 from *V. unguiculata ssp cylindrica* is a homolog of lectin from *Pisum sativum* (garden pea). The study also showed that protease inhibitors with accession numbers of ADB44827 and CBJ34319 are homologs of protease inhibitors from *Mucuna pruriens* (5dss) (Table 1 and Figure 2) while β -amylase from *C. cajan* and legumin from *V. unguiculata* with accession numbers of CAA12395 and AAK61346 respectively were homologs of β -amylase from *Glycine max* (Soybeans) and legumin from *P. sativum* (Garden pea) respectively (Table 1 and Figure 2).

The study showed that chitinase from *P. vulgaris* (Accession number: CAA40474) and *V. unguiculata* (Accession number: CAA61281) have similar structures and considerable sequence conservation (Figure 4 A) with Class IV chitinase from *Z. mays* (PDB: 4MCK) (Figure 2 A) suggesting that they may have similar functions. Binding site analysis of class IV chitinase (4mck) from *Z. mays* shows that some amino acids (Val1, Val2, Ser3, Arg26, Phe29, Leu30, Val33, Phe39, Ala40, His41, Glu45, Lys49, Ile52,) are found at the binding site of a peptidic ligand (Figure 2). Structural alignment revealed that majority of the amino acids at the binding site and other amino acids are conserved (Figure 3 A) among class IV chitinase from *V. unguiculata* (Accession number: CAA61281) and *P. vulgaris* (Accession number: CAA40474). The class IV chitinase from *V. unguiculata* and *P. vulgaris* shows good sequence similarity even though the chitinase from *Vigna unguiculata* was only partially characterized. It has been reported elsewhere [16] that Glu62 in chitinase from *Z. mays* is directly involved in catalysis and that Glu62, Arg177 and Glu165 perform key role in hydrolysis while Ser103 and Tyr106 are involved in substrate binding. Also, study has showed that heveinlike domain in *Z. mays* chitinase is not needed for enzyme activity and that mutation of Glu62 to Gln in class IV chitinase abolished its activity without disrupting substrate binding, demonstrating that Glu62 is directly involved in catalysis [16].

Table 1: Comparative Homology Modeling of the closely related bean Seed Storage Proteins

Sequence	Source	Template	Sequence identity (%)	Sequence similarity	Range	Coverage
AAL23841.1 (trypsin inhibitor)	<i>Phaseolus vulgaris</i>	3RU4(B) (Bowman-Birk serine protease inhibitor from <i>Vigna unguiculata</i>)	77.05	0.58	41-101	0.57
ABD85194.1 (Trypsin inhibitor)	<i>Vigna unguiculata</i>	3RU4(B) (Bowman-Birk serine protease inhibitor from <i>Vigna unguiculata</i>)	77.05	0.58	41-101	0.57
CAA40474.1 (chitinase)	<i>Phaseolus vulgaris</i>	4MCK(A) (Class IV chitinase from <i>Zea mays</i>)	60.20	0.49	72-271	0.72
CAA61281.1 (chitinase)	<i>Vigna unguiculata</i>	4MCK(A) (Class IV chitinase from <i>Zea mays</i>)	58.46	0.49	51-249	0.78
AEW50184.1 (lectin)	<i>Cajanus cajan</i>	1LEM(A) (lectin from <i>Lens culinaris</i>)	98.34	0.61	31-211	0.66
CAM35518.1 (lectin)	<i>Vigna unguiculata</i>	10FS (lectin from <i>Pisum sativum</i>)	93.33	0.59	1-99	0.68
CBJ34319.1 (protease inhibitor)	<i>Vigna unguiculata</i> ssp cylindrical	5DSS(A) (protease inhibitor from <i>Mucuna pruriens</i>) ⁰	41.24	0.39	24-205	0.80
ADB44827.1 (protease inhibitor)	<i>Cajanus cajan</i>	5DSS(A) (protease inhibitor from <i>Mucuna pruriens</i>)	46.20	0.42	17-178	0.73
CAA12395.1 (beta amylase)	<i>Vigna unguiculata</i>	1bfh.1.A (beta-amylase from soybean)	86.06	0.58	7-496	1
AAK61346.1 (legumin seed storage protein)	<i>Cajanus cajan</i>	3ksc.2.B (prolegumin, an 11S seed globulin from <i>Pisum sativum</i> L)	43.20	0.38	7-168	0.49

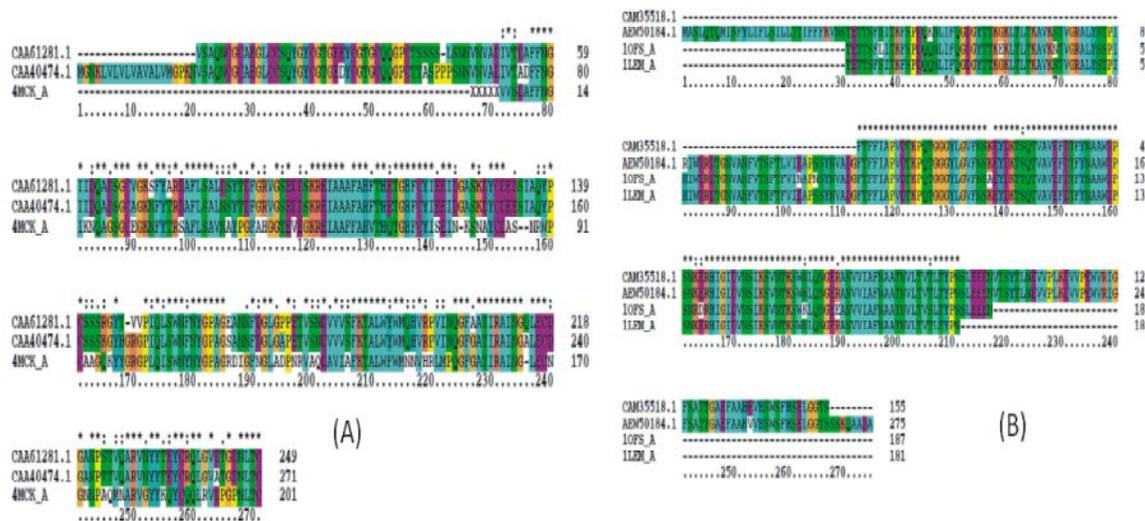


Fig. 3: Sequence alignment of seed storage proteins from using ClustalX-2.1. (A) Alignment of chitinase class 4 (CAA61281.1) from *V. unguiculata*, chitinase, (CAA40474.1) from *P. vulgaris* and chitinase from *Zea mays* (template: 4MCK_A). (B) Alignment of lectin (CAM35518.1) from *V. unguiculata*, lectin (AEW50184.1) from *C. cajan*, lectin from *Pisum sativum* (template: 10fs_A) and lectin from *Lens culinaris* (template: 1lem). Asterisks (*) indicate sequence conservation

Lectin from *C. cajan* (Accession number: AEW50184) is more structurally related to lectin (PDB: 1LEM:A) from *L. culinaris* (lentil) than lectin (PDB: 10FS:A) from *P. sativum* (garden pea) while lectin from *V. unguiculata ssp cylindrical* (Accession number: CAM35518) is more structurally related to lectin from *P. sativum* (garden pea) than that from *L. culinaris* (lentil). The lectins from *C. cajan* and *V. unguiculata ssp cylindrical* showed high degree of sequence conservation with lectin (PDB: 10FS:A) from *P. sativum* (Figure 3 B). The high structural similarity (Figure 2 B), sequence conservation (Figure 3 B) and sequence identities (98.34 % and 93.33 %) (Table 1) of the studied lectins suggests that they have similar function. Binding site analysis of lectin (10fs and ILEM) from *P. sativum* and *L. culinaris* shows that some amino acids (Ala80, Asp81, Phe123, Tyr100, Ile144, Ser146, His136, Asp121, Trp128, Pro130, Glu119, Asp129, Asn125) are found at the binding site of a sucrose and glucose (Figure 2 B). Structural alignment revealed that majority of the amino acids at the binding site and other amino acids are conserved (Figure 2 B) among lectin from *C. cajan*, *V. unguiculata ssp cylindrica*, *P. sativum* and *L. culinaris*. Binding site analysis of β -amylase (1bfn:A) from *G. max* shows that some amino acids (Tyr192, Gln194, Trp301, Trp198, His300, Gln351, Met346, Cys343, Arg385, Glu350, Leu419, Thr342, Leu383, Ala382, Pro352, Pro384) are found at the binding site of a β -D-glucose (Figure 4 A).

Sequence alignment revealed that majority of the amino acids in β -amylase (CAA12395.1) from *V. unguiculata* and β -amylase (1bfn_A) from *G. max* is conserved (Figure 4 A). This suggests that both proteins are homologs of each other. Also, some amino acids in trypsin inhibitor (ABD85194.1) from *V. unguiculata*, trypsin proteinase inhibitor (AAL23841.1) from *P. vulgaris* and Bowman-Birk serine protease inhibitor from *V. unguiculata* (template: 3RU4_B) are conserved (Figure 5 B).

Also, the present study showed that protease inhibitors from *C. cajan* (Accession number: ADB44827) and *V. unguiculata ssp cylindrica* (Accession number: CBJ34319) have similar structure with protease inhibitor (MP-4) from *M. pruriens* (PDB: 5DSS) (Figure 6 B). Traditional medicine suggests that seeds of *M. pruriens* can provide protection against the effects of snakebite [6]. It has been reported elsewhere [6] that MP-4 contributes significantly to snake venom neutralization activity of *M. pruriens* seeds through indirect antibody-mediated

mechanism and it belongs to Kunitz-type protease inhibitor (KTPI) family. Therefore, the protease inhibitors from *C. cajan* and *V. unguiculata ssp cylindrical* may contribute to snake venom neutralization activity through indirect antibody-mediated mechanism just like MP-4 in *M. pruriens*.

Sequence alignment also revealed that some of the amino acids in Kunitz-type protease inhibitor (CBJ34319.1) from *V. unguiculata*, protease inhibitor (ADB44827.1) from *C. cajan* and protease inhibitor from *M. pruriens* (template: 5dss) are conserved (Figure 7). Also legumin (AAK61346.1) from *C. cajan* and prolegumin (3ksc_B) from *P. sativum* showed some level of sequence conservation (Figure 7).

It has been reported that monomeric proteins in beans appear to maintain their genetic and phenotypic similarities [17]. Also, it has been reported elsewhere [18] that water soluble proteins in *V. unguiculata ssp sesquipedalis*, *V. unguiculata unguiculata*, *C. cajan* and *M. pruriens* are closely related. The monomeric and water soluble proteins reported elsewhere [17, 18] are probably chitinase, lectin, protease inhibitor, trypsin inhibitor, β -amylase and/or legumin reported in the present investigation. Previous study had shown that Phaseolus contain a family of related lectins and lectin-like proteins that are associated with antibiosis activity against seed storage pests [5]. The present study showed that lectins from *C. cajan* and *V. unguiculata ssp cylindrical* showed high degree of structural similarity, sequence conservation and sequence identities with lectin from *P. sativum* suggesting that they have similar function. Lectins in *C. cajan* and *V. unguiculata ssp cylindrical* may play similar role with that in *Phaseolus*.

The estimated absolute model quality by comparison with non-redundant set of PDB structures of the models is shown in the Figure 8. The QMEAN (Qualitative Model Energy ANalysis) Z-score of the models are less than 1 (Z-score < 1) (Figure 8). According to Benkert *et al.* [15] 'good' models have average QMEAN Z-score of -0.65 which is comparable to experimental structures while 'medium' quality models have mean Z-score of -1.75.

Legumin and protease inhibitor with sequence identities of less than 50% (Table 1) and higher QMEAN Z-score are not reliable/quality models. However, the non-reliability of the models could be attributed to the fact the proteins sequences used were only partially characterized.

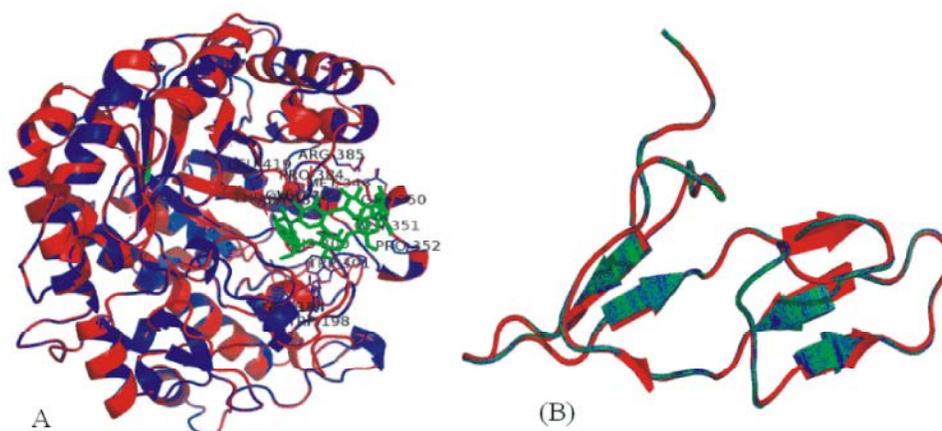


Fig. 4: Structural Alignment of seed storage proteins using PyMol. (A) β -amylase: red (CAA12395) is from *V. unguiculata*, blue is template (1bfn_A) is from *G. max*, green is β -D-glucose. (B) trypsin inhibitor: green (AAL23841) is from *P. vulgaris*, blue (ABD85194) is from *V. unguiculata*, red (3RU4:B Bowman-Birk serine protease inhibitor from *V. unguiculata*) is template

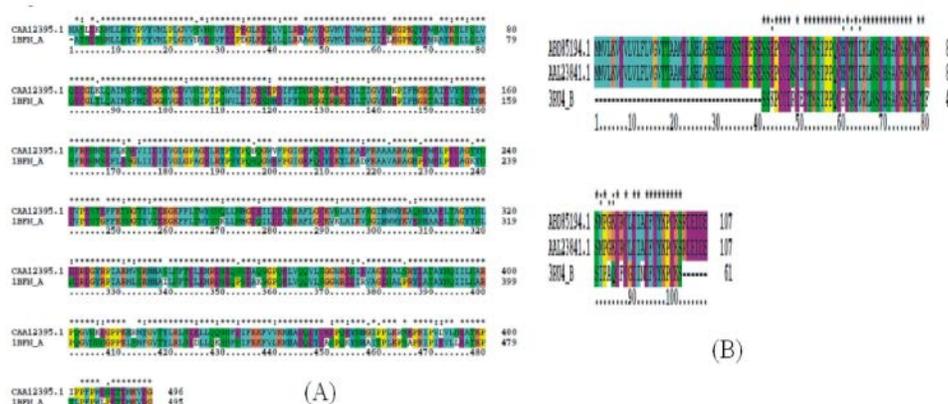


Fig. 5: Sequence Alignment of seed storage proteins using ClustalX-2.1 (A) Alignment of β -amylase (CAA12395.1) from *V. unguiculata* with β -amylase (template: 1bfn_A) from *G. max*. (B) Alignment of trypsin inhibitor (ABD85194.1) from *V. unguiculata*, trypsin proteinase inhibitor (AAL23841.1) from *P. vulgaris* and bowman-birk serine protease inhibitor from *V. unguiculata* (template: 3RU4_B). Asterisks (*) indicate sequence conservation

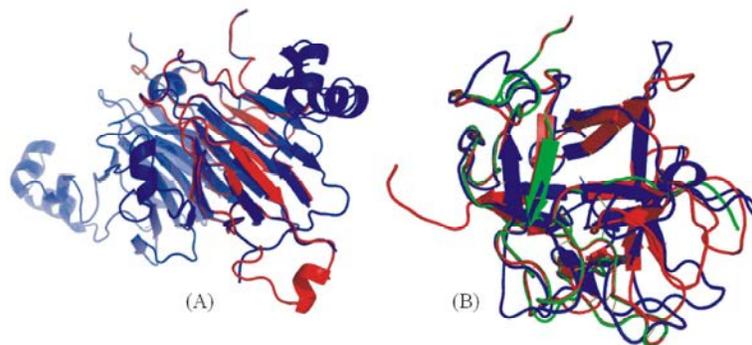


Fig. 6: Structural Alignment of seed storage proteins using PyMol (A) Legumin: red (AAK61346) is from *C. cajan*, blue is prolegumin (3ksc) is from *Glycine max* is the template. (B) protease inhibitor: green (ADB44827) is from *Cajanus cajan*, blue (CBJ34319) is from *V. unguiculata ssp cylindrica*, red (5DSS: protease inhibitor (MP-4) from *M. pruriens*) is template

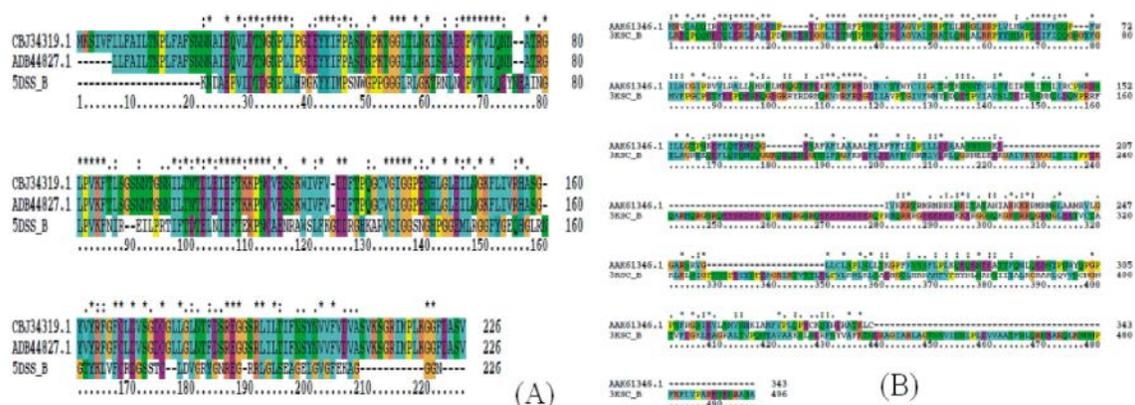


Fig. 7: Sequence alignment of seed storage proteins using ClustalX-2.1 (A) Alignment of kunitz-type protease inhibitor (CBJ34319.1) from *V. unguiculata*, protease inhibitor (ADB44827.1) from *C. cajan* and protease inhibitor from *M. pruriens* (template: 5dss). (B) Alignment of legumin (AAK61346.1) from *C. cajan*, with prolegumin (template: 3ksc_B) from *P. sativum*. Asterisks (*) indicate sequence conservation

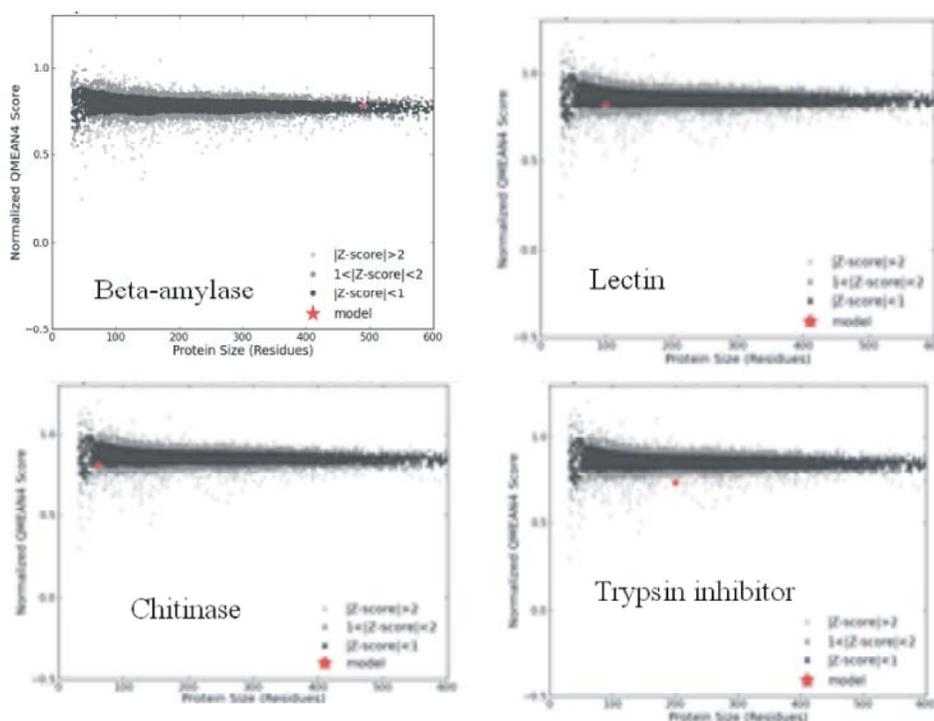


Fig. 8: Comparison with non-redundant set of PDB structures of the models

CONCLUSION

Beta-amylase and other seed storage proteins in *Phaseolus vulgaris*, *Vigna unguiculata* and *Cajanus cajan* which are associated with pests and pathogens control are more conserved and structurally related than the other seed proteins. Also, some of the amino

acids at the binding site and other amino acids in the bean storage proteins are conserved. The high structural similarity, sequence conservation and identities of the studied beans proteins suggest that they have similar functions in the species. This could be exploited for development of pest and/or pathogen resistant bean species.

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