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# *IN SILICO* STUDIES ON STRUCTURE-FUNCTION OF DNA GCC- BOX BINDING DOMAIN OF *BRASSICA NAPUS* DREB1 PROTEIN

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**Abstract**

DREB1 is a transcriptional factor, which selectively binds with the promoters of the genes involved in stress response in the plants. Homology of DREB protein and its binding element have been detected in the genome of many plants. However, only a few reports exist that discusses the binding properties of this protein with the gene (s) promoter. In the present study, we have undertaken studies exploring the structure-function relationship of *Brassica napus* DREB1. Multiple sequence alignment, protein homology modeling and intermolecular docking of GCC-box binding domain (GBD) of the said protein was carried out using atomic coordinates of GBD from *Arabdiopsis thaliana* and GCC-box containing DNA respectively. Similarities and/or identities in multiple, sequence alignment, particularly at the functionally important amino acids, strongly suggested the binding specificity of *B. napus* DREB1 to GCC-box. Similarly, despite ~56% sequence homology, tertiary structures of both template and modeled protein were found to be extremely similar as indicated by root mean square deviation of 0.34Å. More similarities were established between GBD of both *A. thaliana* and *B. napus* DREB1 by conducting protein docking with the DNA containing GCC-box. It appears that both proteins interact through their β-sheet with the major DNA groove including both nitrogen bases and phosphate and sugar moieties. Additionally, in most cases the interacting residues were also found to be identical. Briefly, this study attempts to elucidate the molecular basis of DREB1 interaction with its target sequence in the promoter.

## Introduction

Plant growth and consequently yield is adversely affected by certain abiotic stresses such as salinity, drought and temperature fluctuations. The ability to achieve optimal agricultural output is a serious challenge due to increasing environmental stresses. Indeed the expected yield of crop plants can be reduced up to 70% due to these factors. In this regard water associated stress, for instance drought, salinity and temperature severity are considered among the most damaging (Knight & Knight, 2001; Agarwal *et al.,* 2006). Plants respond and acclimatize to these stresses by developing defensive arsenals and strategies at anatomical, physiological, biochemical and genetic levels (Shinozaki *et al.*, 2003). At the molecular level, several signaling pathways are known to regulate stress responses in plants (Knight & Knight, 2001; Chen *et al.*, 2002). The effective usage of transcriptional analysis has allowed classifying the stress-associated genes into two broad categories (Lin *et al.*, 2008; Davey *et al*., 2009). The first includes functional proteins like membrane channel proteins, detoxifying enzymes and macromolecules protecting proteins. The second and equally significant category includes regulatory proteins and/or transcriptional factors, proteinases and protein kinase (Riechmann *et al*., 2000; Seki *et al*., 2001; Abe *et al*., 2003). Several transcriptional factors are known in this connection for example bZIP, MYC, MYB and DREB. Transcription factors, encoded by *dreb* genes, are induced by cold and water stress, and are found to bind with DRE promoter element of stress related genes triggering their expression. This cis-acting DNA (DRE) element is present in the promoters of genes such as *rd17*, *rd29A*, *cor15a*, *cor6.6*, *kin1* and *erd*. The binding of DREB initiates synthesis of gene products implicated in plant acclimation response to low temperature and water stress (Gilmour *et al.*, 1998).

The DREB transcription factors have been divided into two classes DREB1 and DREB2 based on their involvement in signal transduction pathways under low temperature and dehydration or high-salinity stress, respectively. The proteins of DREB1-type are constitutively active in plants but that the DREB2-type proteins possibly require alteration in response to stress for its activation in plants (Yamaguchi-Shinozaki & Shinozaki 1994). The Dehydration Responsive Element (DRE), which arguably has a core sequence TACCGACAT, is recognized by proteins of the DREB subfamily (Yamaguchi-Shinozaki & Shinozaki 1994, Stockinger *et al*., 1997). The sequence CCGAC inside the DRE element is the minimum sequence motif for binding, and C4, G5, and C7 are essential for specific interaction (Hao *et al*., 2002, Sakuma *et al*., 2002). Additionally, the DNA-binding specificity of *Arabidopsis* DREBs is well known. It has been shown that both DREB1 and DREB2 specifically bind to six nucleotides (A/GCCGAC) of DRE. This consensus sequence is generally referred as GCC-box, thus strongly suggesting that DREB proteins contain GCC-box binding domain (Agarwal *et al*., 2007).

To understand the molecular mechanism of target recognition and to envisage target genes for transcription factors at the genome level, it is imperative to analyze the relationship between the structure and function (specificity) of transcription factors (Garg *et al*., 2008). In the present study, we have undertaken the protein homology modeling of DREB1 protein from *Brassica napus* to study its structural attributes. Furthermore, the

protein has also been docked with the DNA double helix having GCC-box to elucidate the residues involved in the Protein-DNA interaction. To the best of our cognizance, this is the first report regarding structure-function aspects of *B. napus* DREB1 using protein homology modeling and protein-DNA docking strategies. We believe that the present findings will illustrate more insights into the structure-function role of DREB1 protein in molecular terms.

## Materials and Methods

**Multiple sequence alignment:** Primary structure sequences of DREB1 protein of *Brassica napus* (accession number ABB17252) and GCC-box binding domain from *Arabidopsis thaliana* (PDB code 1gcc) were retrieved from NCBI (National Center for Biotechnology Information) data bank (Wheeler *et al*., 2005). Primary and tertiary structure homologs of the mentioned protein were found using program FASTA and BLAST (Altschul *et al*., 1997). Multiple sequence alignment was conducted by default parameters of software Clustal X (Thompson *et al*., 1997). After some non-redundant manual modification, alignment file was analyzed using GeneDoc (Nicolas *et al*., 1997) and visualized by CLC Sequence Viewer 6.0.2 (http:// www.clcbio.com/index.php?id=28).

**Homology modeling:** As templates, the atomic coordinates of GCC-box binding domain from *Arabidopsis thaliana* complexed with GCC-box containing double helix DNA (PDB code 1GCC) (Allen *et al*., 1998) were retrieved from Protein Data Bank (PDB) (Berman *et al*., 2000). The tertiary structure models of DREB1 of *Brassica napus* were constructed using Geno3D (Combet *et al*., 2002) and SWISS-MODEL (Schwede *et al*., 2003) with the manual input of PDB code of the template.

**Tertiary structure analysis:** The constructed models of DREB1 from *B. napus* were viewed by Swiss PDB viewer (Guex & Peitsch, 1997) and Accelrys Discovery Studio visualizer 2.0 (http:/accelrys.com/products/ discovery studio/). The structural and thermodynamic stability of all models were verified using Swiss-PDB viewer, PROCHECK, Whatcheck (Laskowski & Kato, 1980), ANOELA (Melo & Feytmans, 1998) and Verify3D (Elsenberg *et al*., 1997). Folds in the modeled protein were recognized from 3D-PSSM algorithm (Kelley *et al*., 2000).

**Docking studies:** The selected model of DREB1 was docked against the GCC-box containing double helical DNA using the docking simulated program BIGGER assisted with program CHIMERA (Palma *et al*., 2000). One thousand models were constructed with defined global scoring covering electrostatic, hydrophobic; solvation energy and side chain contacts attributes, hydrophobic and electrostatic restrains. Out of these the best hydrophobic and electrostatic models were chosen & find model with reference to global score was selected for detailed analysis.

## Results and Discussion

**Multiple sequence alignment:** DREB1 is 214-residue long protein from *B. napus*. Its GCC-box binding domain (GBD) ranges from Tyr54 to Asp111. Multiple sequence alignment of this GBD with that of *A. thaliana* GBD suggested approximately 56% identity with almost equal distribution of homology along the protein (Fig. 1). In addition to this, functionally important residues of GBD (GCC-box binding amino acids) were found to be almost identical both in *A. thaliana* and *B. napus*. A consensus sequence of GCC-box i.e., AGCCGCC, to which the GBD binds, has been reported in the promoter region of the genes involved in responding to a variety of biotic stresses (Ohme-Takagi & Shinshi, 1995; Chen *et al*., 2008). For instance GmERF3 gene, an AP2/ERF type transcription factor and ethylene-responsive element binding proteins (EREBPs) from tobacco and AtERF-1-4 and AtEBP from *A. thaliana* both tend to bind with GCC-box (Ohme-Takagi & Shinshi, 1995; Buttner & Singh, 1997). The protein region of roughly 60 residues long was suggested to bind with this GCC-box and so named as GCC-box binding domain (GBD) (Allen *et al*., 1998; Agarwal *et al*., 2006). Divergent proteins in a wide range of plants contain the GBD domain (Elliot *et al*., 1996; Klucher *et al*., 1996; Wilson *et al*., 1996; Okamuro *et al*., 1997; Chen *et al*., 2005). Similarly, ERF proteins originate from the APETALA (AP2) or ethylene responsive element binding protein (EREBP) transcription factors. DREB1/C repeat binding factor (CBF) genes, which are stimulated by cold stress (Fowler & Thomashow, 2002, Ito *et al*., 2006), to provide tolerance to cold stress in various plant species. This has been shown in maize, rice, barley, wheat, soybean and *Brassica,* where they also contain GBD. Moreover, both DREB1/CBF and DREB2 genes have similar sequences at AP2 domain and these bind to the same DRE sequence (Liu *et al*., 1998, Gilmour *et al*., 1998). This suggests an almost ubiquitous distribution of GBD domain containing protein among the plant kingdom. To date no strong homolog has been detected among animal and fungal proteins (Allen *et al*., 1998), but a comparative sequence analysis conducted by Rivero *et al*., (2005) may suggest presence of evolutionary links of GBD in different animals and fungal species. Studies conducted on *A. thaliana* GBD stipulated that residues like Arg147, Gly148, Arg150, Arg152, Trp154, Lys156, Arg162, Arg170, Trp172, Thr175 and Tyr186 have been found involved in the binding of GBD containing protein with DNA GCC-box (Allen *et al*., 1998). Except Ser62 and His93, which replaced Trp154 and Tyr186 of *A. thaliana* GBD respectively; the earlier mentioned residues with some spatial differences were found to be identical in the GBD of *B. napus* DREB1 (Fig. 1). This implies a common mechanism of action and Protein-DNA interaction. However, the holistic homology between GBD of *A. thaliana* and *B. napus* (~56%) may possibly point towards significant conformational discrepancies in both these molecules and consequently entails their different mechanistic role. In order to verify/reject this notion, protein homology modeling and Protein-DNA docking studies were conducted.

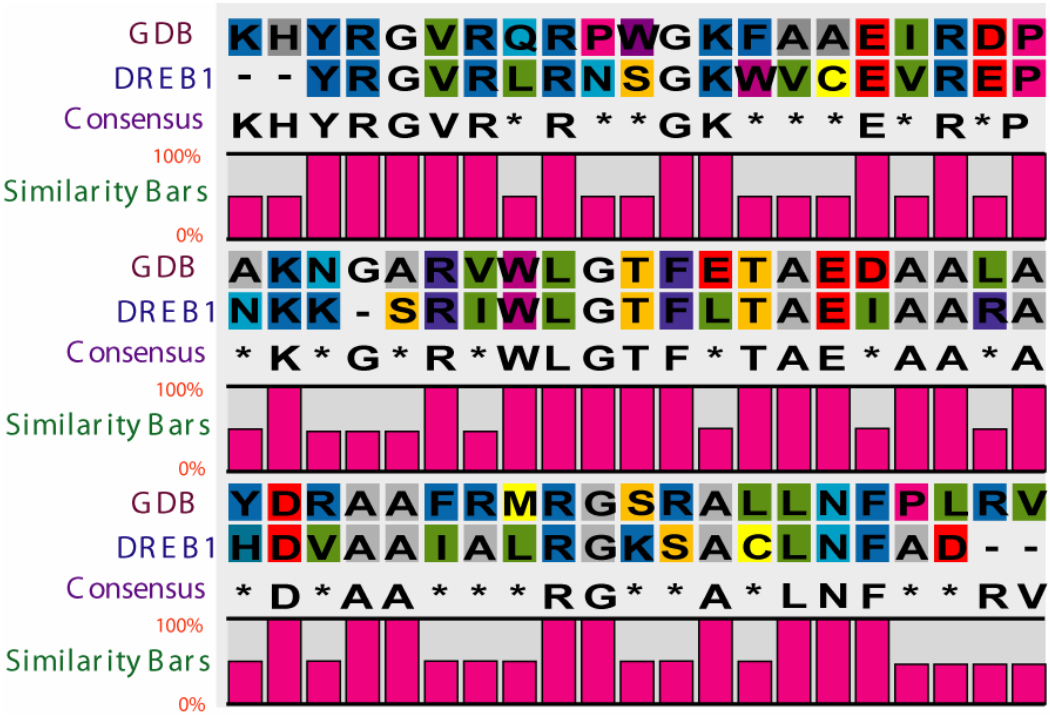


Fig. 1. Multiple sequence alignment of aminoacids of template and DREB GCC-binding domain (GDB). Consensus sequence and conservation percentage histogram is represented at the bottom of the alignment.

**Overall tertiary structure:** Holistically, the modeled tertiary structure of *B. napus* DREB1 GBD is structurally very similar to GBD of *A. thaliana*. Both template and predicted structures comprise on three stranded anti-parallel β-sheet followed by α-helix and relatively unstructured C terminal. In *B. napus,* three stranded anti parallel β-sheet of DREB GBD contains strand1 (Val57-Asn61), strand2 (Lys63-Arg70) and strand3 (Arg77-Phe83) while α helix ranges from Ala83 to Arg101 residues (Fig. 2). With the distance criteria of 2.5Å, 33 hydrogen bonds were found in the protein, which may be involved in the establishment and consequently stabilization of the tertiary structure of the protein. As found in the *A. thaliana* GBD NMR based structures (Allen *et al*., 1998), the *B. napus* DREB1 GBD was also found to be stabilized by a large number of hydrophobic interactions among the residues of corresponding bio-physicochemical properties. Similarly, the geometry of α-helix relative to β-sheet was established with relatively larger number of Ala residues in the former and more of Phe and Val in the later, which possibly holds the α-helix at four corners. Intriguingly, this is unlike most other proteins where α-helix is tilted with respect to β-sheet (Janin & Chothia, 1980). Similar to GBD of *A. thaliana* (Allen *et al*., 1998) and modeled structure the direction of N to C terminal of α-helix was found almost parallel to strand 2 of β-sheet. In short, despite a relatively less primary structure homology between GBD of *A. thaliana* and *B. napus* DREB1, their tertiary structure resembled to each other considerably as suggested by the root mean square deviation (RMSD) of 0.34 Å (Fig. 2). Such differences and/or similarities among functionally related proteins have also been noticed in earlier studies conducted on DNA-Photolyase (Hussain *et al*., 2009). The values also suggest to the fidelity of the modeling strategies used in the present study. In addition to this around 95% residues of the modeled protein (*B. napus* DREB1 GBD) were found in the acceptable constraint of Ψ and Φ angles in the Ramachandran plot (Wilson *et al*., 1998) and bear the free energy of –2766.856 KJ/mole, suggesting the structural and thermodynamic stability of the proposed structure of the DREB1.

**Fold recognition:** As anticipated, most of the folds present in the B. napus DREB1 GBD were similar to folds present among other structured DNA/RNA binding proteins. However, interestingly, some of the folds showed resemblance to proteins that are evolutionary unrelated like bunger toxin (d2abxa; snake venom protein) and viral protein (Clcwxa; Hepatitis C virus).

**Protein-DNA docking:** Electrostatically, DNA being a negatively charged biomolecule binds with the protein region(s) where the positive residues like Lys, Arg and/or His are concentrated. The known binding site of GBD of *A. thaliana* strengthens this notion (Allen *et al*., 1998). Similar to this, it was found that GBD of *B. napus* DREB1 also possessed same electrostatic potential and surface topology as noticed in GBD of *A. thaliana*. However, relatively more positive charge has been noticed in GBD of *B. napus* DREB1 as compared to the same version of protein found in *A. thaliana* (Fig. 3). This may point toward stronger binding of *B. napus* transcriptional factor with promoter (GCC-box) of genes in comparison to *A. thaliana*. The intermolecular docking studies revealed that like GBD of *A. thaliana* (Allen *et al*., 1998), *B. napus* DREB1 GBD also binds with major groove of DNA *via* its three stranded anti parallel β-sheet. Similarly, the N to C terminal of the protein corresponds to the 5' to 3' terminal of the DNA coding strand. Electrostatic surface-to-surface contacts reveal complete accommodation of both molecules into each other (Fig.

4). Generally the *Protein-DNA* interactions are established through α-helices of zinc finger containing proteins (Dutnall *et al*., 1996; Tan *et al*., 2003), however, it has appeared that GBD and a few other DNA interacting plant proteins may exploit their β-sheet(s), for example MetJ and Arc repressor proteins (Breg *et al*., 1990; Somers & Philips, 1992; Raumann *et al*., 1994; Mazarel *et al*., 2002). However, an in-depth analysis suggests that β-sheets in these proteins form dimeric interface and their DNA sequence specificity is always pallindromic in nature. Conversely, the GBD of *B. napus* DREB1 has three stranded anti-parallel β-sheet and monomeric and it interacts with non-pallindromic sequence of DNA, this is similar to what was found by earlier studies of Allen *et al*., (1998). Furthermore, the MetJ-Arc type repressor recognized six consecutive base pairs in their target DNA (Suzuki, 1995) while the understudy protein recognized nine consecutive base pairs (Jiang *et al*., 1996; Ouellet *et al*., 1998).

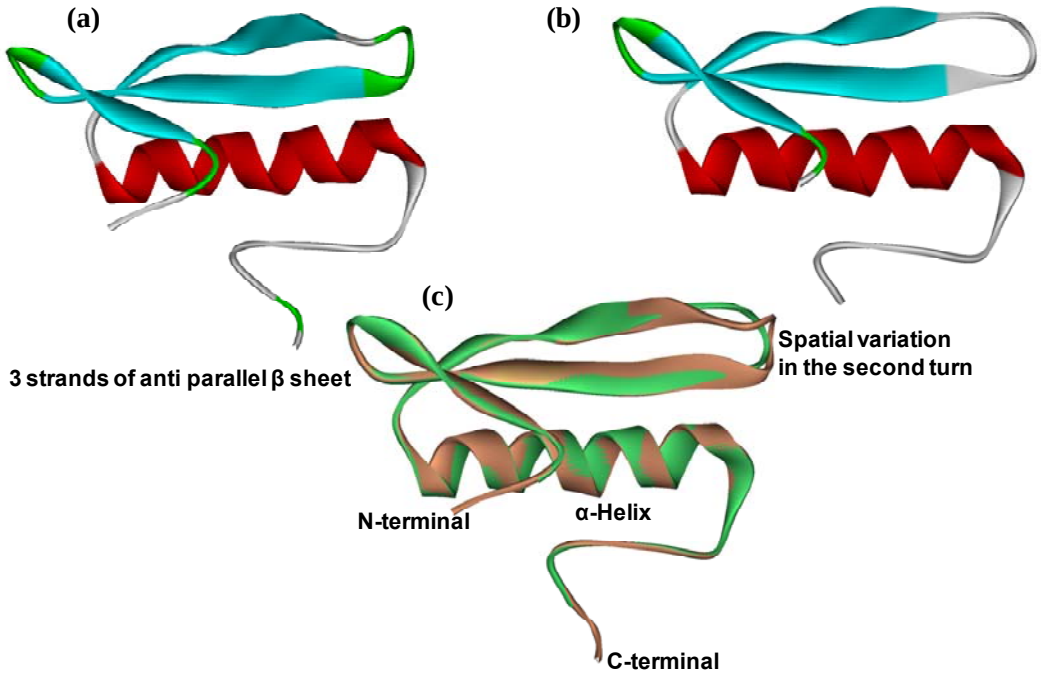


Fig. 2. Tertiary Structure of GDB: Tertiary helical structure of (a) template GDB and (b) modeled DREB1 GDB. (c) Superimposition of both the structures suggests significant conservancy in the tertiary structures of template (brown) and DREB1 (green) GDB except at second turn. Important structural aspects are annotated in the superimposed structures. Three strands of anti parallel β sheet are respectively represented by order with blue, brown and red arrows.

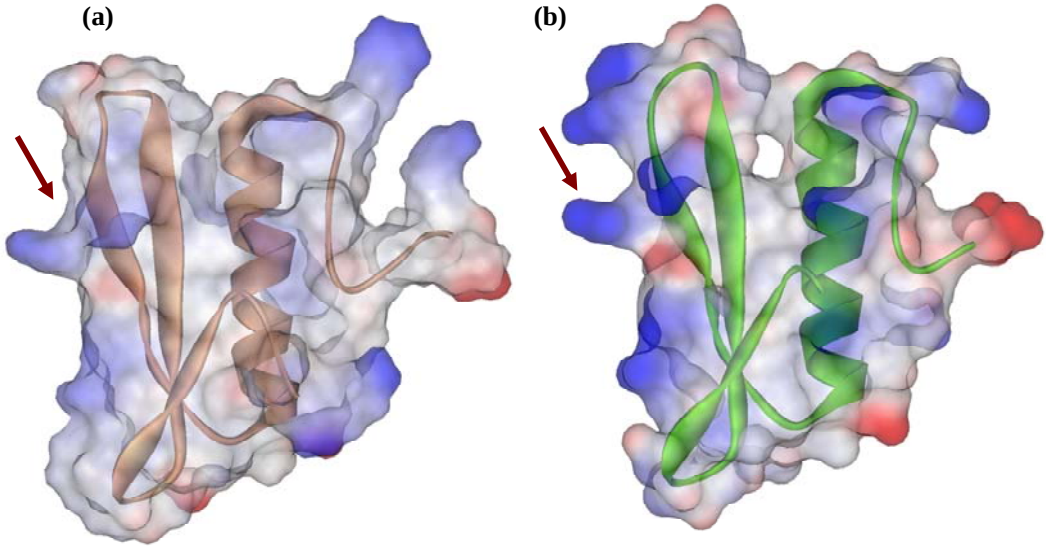


Fig.3. Electrostatic surface potential of GDB of (a) template and (b) modeled DREB1 GDB. DNA binding site is indicated with purple arrows. Note the presence of more positive charges at DNA binding sites of DREB1 GDB as compared to template GDB.

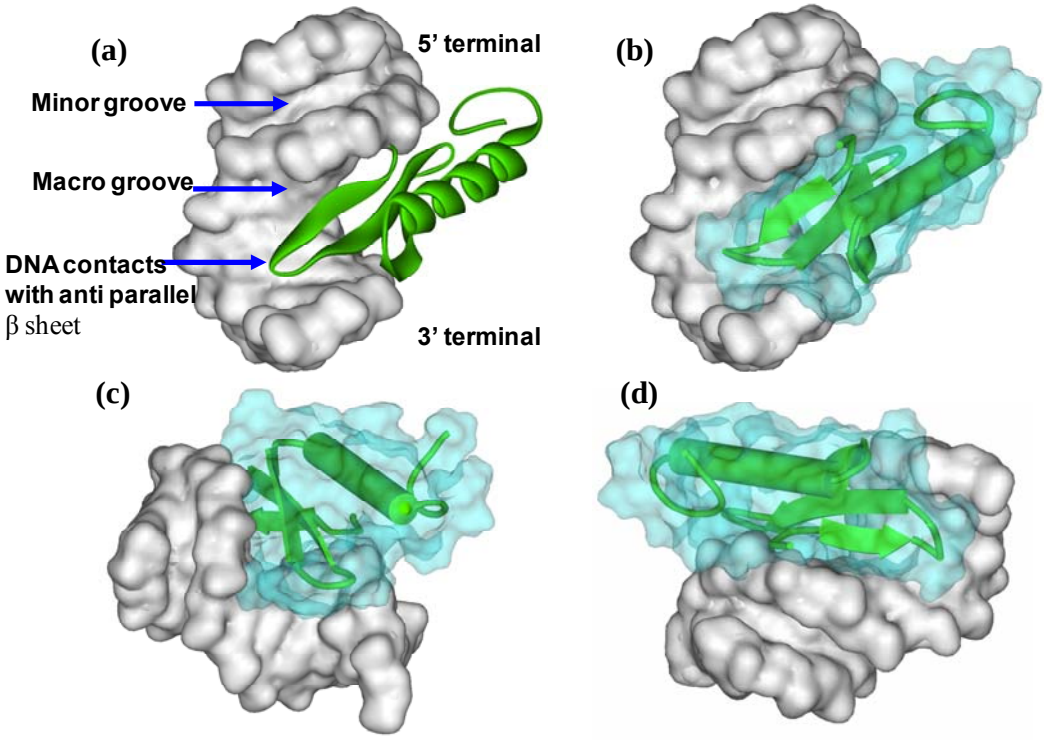


Fig. 4. Docking of DREB1 GDB with DNA double helix with conserved AGCCGCC box. (a) Three anti parallel β sheets binds with the macrogroove of DNA with N-terminal of protein corresponding to 5' terminal of DNA. (b, c & d) Different orientation of surface to surface contact with protein and DNA. Schematic representation of protein is also illustrated with helix and β sheet represented by cylinder and directional arrows respectively.

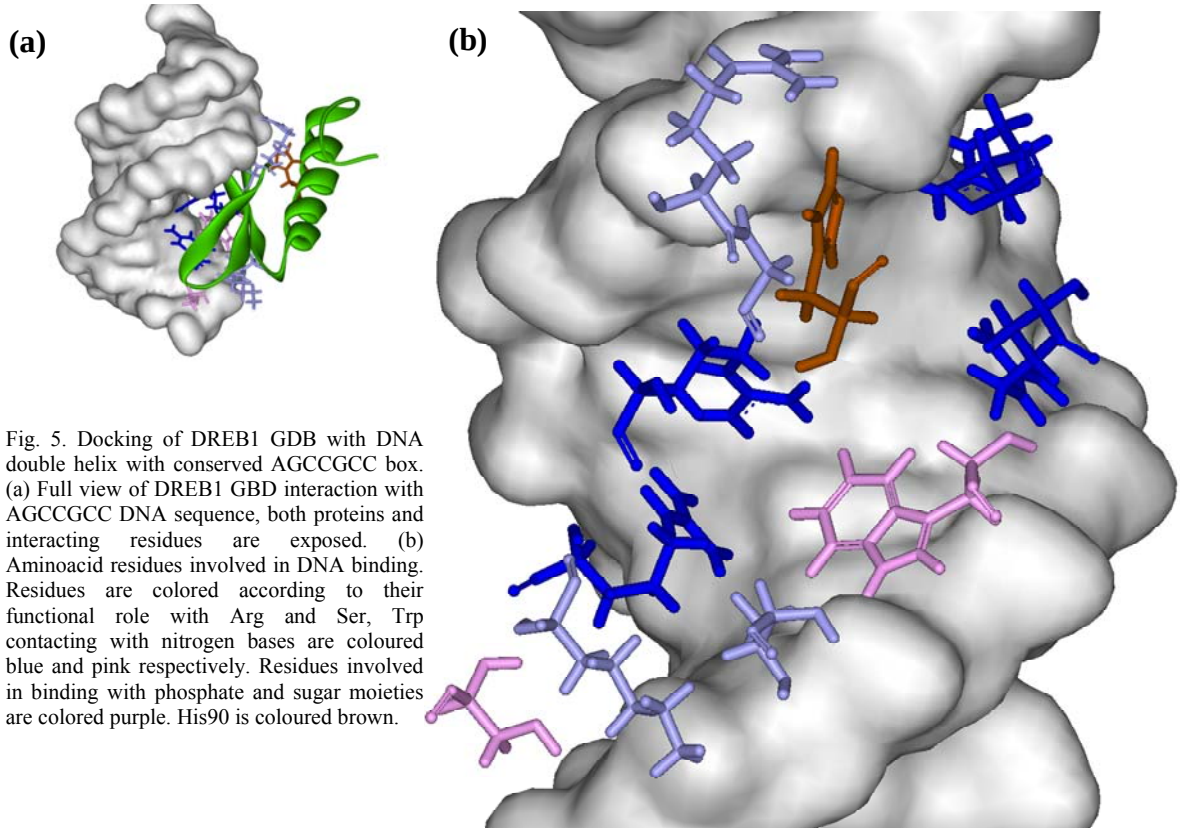


Fig. 5. Docking of DREB1 GDB with DNA double helix with conserved AGCCGCC box. (a) Full view of DREB1 GBD interaction with AGCCGCC DNA sequence, both proteins and interacting residues are exposed. (b) Aminoacid residues involved in DNA binding. Residues are colored according to their functional role with Arg and Ser, Trp contacting with nitrogen bases are coloured blue and pink respectively. Residues involved in binding with phosphate and sugar moieties are colored purple. His90 is coloured brown.

More precisely, the guanidyl groups of four Arg residues were found to establish hydrogen bond with five guanine bases (Fig. 5); Arg58 to G20, Arg60 to G5, Arg70 to G17 and Arg77 to G8. However, the stem architecture of the said residues binds with the cytosine and adenine. Ser62 and Trp79 were found to establish hydrophobic contacts with the T3 and A4, and G5 and C6 respectively. It is important to note here that in the *A. thaliana* GBD, the Ser62 was found replaced by Trp154 and is known to interact with similar bases T3 and A4 (Allen *et al*., 1998). Overall, the residues interactions directly cover six base pairs in the conserved AGCCGCC sequence validating the presence of GBD in the *B. napus* DREB1. In addition to the nitrogen bases, except Arg60, all interacting Arg and Trp also establish ionic or hydrophobic interaction with the phosphate group or sugar moiety of the DNA respectively (Fig. 5). In one half of the protein-DNA complex, residues present in strand 3 contacts with the bases of coding strand, while amino acids on strand 2 establish their links with the complementary strand, while strand 1 residues interact with both DNA strands. The over all structure of the DNA in the complex is more or less similar to B allomorph of the DNA, however, a slight kink was noticed around the major groove at the sequence permutation of CG. Additionally, certain other residues also interact with sugar phosphate backbone of DNA molecule, for instance Arg55, Gly56, Lys64, Thr82 and His93. It is interesting to note here that all the earlier and later mentioned residues were located in three stranded anti parallel β-sheet except His93 which was found in α-helix and found to be the only residue of α-helix which has some interaction with the GCC-box (Fig. 5). In contrast to some experimental work suggesting the presence of Val and Glu for the effective interaction of β-sheet with DNA (Sakuma *et al.*, 2002), no such interaction has been observed in this study. It could be possible that these residues may be involved in the structural stability of the molecule, any alteration in those may cause change in the configuration of the protein and subsequently the truncation of its function.

## Conclusion

Quite opposite to the values indicated by primary structure sequence alignment, the proposed structure of *B. napus* DREB1 GBD is significantly conserved with the GBD found in *A. thaliana*. Additionally, involvement of more of less identical residues and intermolecular docking studies could be plausibly inferred in terms of similar mode of action of both proteins. However, more concentrated positive residues may result in stronger binding of DREB1 with its respective promoter as compared to GBD of *A. thaliana*. Further intermolecular docking studies using different permutations of DNA are underway and will be reported shortly.

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