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Identification of the likely orthologues of RCD1 within the plant family Brassicaceae

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Abstract

RCD1, Radical-induced Cell Death 1, is a signal transduction factor binding protein that gateways a myriad of developmental and stress-related pathways. It was first reported in the wild plant *Arabidopsis thaliana*. *Brassica napus* is a cultivated member of the family Brassicaceae, in which the presence of this gene was reported. Using the homology data of these two family-related species, gene for this protein was mined within the genomes of *Brassica carinata*, *Brassica juncea* and *Brassica oleracea*, using sets of degenerate primers designed on homologous portions of the *A. thaliana* and *B. napus* orthologues. The newly identified sequences were then compared and studied using *in-silico* means and their 3D structures were modelled for having an estimate on their functions. Results demonstrate intergeneric conservation of this protein's domains on structural and functional levels. The newly found orthologues show potential to be regulated under salinity and oxidative stresses apart from being involved in several developmental stages. These homologues are in-stable *in-vivo* and bear motifs for binding a wide-variety of transcription factors. The structure superimposition studies suggest that these *Brassica* orthologues bear the WWE domains having transferase activity, the fact that can dramatically increase the survival of these agriculturally important crop plants amid the adverse environmental conditions.

Keywords: RCD1, SRO, PARP, WWE, RST, *de-novo* gene finding

INTRODUCTION

Genes of importance for the survival of plants are retained within their genomes as a function of selective evolution. Obsolete deletions or complete absence of such important genes make it difficult for plants to thrive (Darwin and Bynum, 2009). Therefore, the regions of evolutionary importance are found conserved both at genetic and genomic levels, unless the pressures of artificial selection have acted otherwise (Prakash, 2000). These genomic regions encode transcription factors, transcription factor binding elements and comprise constitutive coding regions. Upon perception of a change in external environment; such as an increased heat, cold, salinity, drought or pathogen attack; through the generated ROS (Reactive Oxygen Species) the plants' internal systems are activated for synergistic or antagonistic reactions (Rejeb *et al.,* 2014). The ultimate outcome of such reactions is gene activation or suppression, leading to the transcriptional reprogramming of cells (Mur *et al.*, 2006). An important player in such pathways is RCD1 (Radical-induced Cell Death 1) that has been implicated in both abiotic as well as biotic stress responses (Brosché *et al.*, 2014). Apart from its role in stress responses, it has also been demonstrated to be important for the proper development of a plant (Jaspers *et al.*, 2009; Teotia and Lamb, 2009). Presently understood function of its protein product is of a transcriptional activator that responds to the elevated levels of certain hormones (Salicylic acid, Methyl Jasmonates,

etc) to bring about responses such as shade avoidance and transcriptional reprogramming of cells, while its basal levels are implicated in acting as suppressors of gene expression (Overmyer *et al.*, 2000; Ahlfors *et al.*, 2004; Brosché *et al.*, 2014; Wirthmueller *et al.*, 2017). The RCD1 protein accomplishes these goals by primarily binding to a large array of transcription factors through its N-terminal RST domain (Katiyar-Agarwal *et al.*, 2006; Jaspers *et al.*, 2009; You *et al.*, 2014) and this binding is implicated in regulating the transcriptional control of more than 500 differentially regulated genes (Jaspers *et al.*, 2009).

Since the first report of its presence within *A. thaliana*, the occurrence of this gene has been reported from *Selagenella moelendorfii* and presently in all explored land plants' genomes (Belles-Boix *et al.,* 2000, You *et al.*, 2014). However, its extensive characterization has been carried out only in the model plant of *A. thaliana* where it has been found to be the most active member of SRO family. The members of this family harbour a central PARP domain and an N-terminal RST domain, while only RCD1 and its closest paralogue SRO1 bear an additional domain named WWE (Ahlfors *et al.*, 2004; Jaspers *et al.*, 2010). The SRO members with PARP-RST structures have been reported only in the plant family of Brassicaceae (Jaspers *et al.*, 2010) while another category of such orthologues is LROs that harbour WWE and RST domains. The LROs have yet been reported only in case of the plant family Fabaceae (Siddiqua *et al.*, 2016).

The Brassicaceae plant family houses several important food crops that include *Brassica juncea*, *B. napus*, *B. oleraceae* and *B. carinata*. Varieties of these plants are cultivated for obtaining oil and vegetables. In 2010, 76 million tons of crops belonging to this genus were produced with net worth of 14.85 billion USD (http://faostat.fao.org/). Existence of RCD1 within members of *Brassica* genus opens an area for manipulation of this gene for the modulation of stress tolerance characters of these cultivated plants. From this genus, studies on RCD1 homologue from *B. napus* has been carried out (Anjum *et al.,* 2015). Here we report the existence of members of the WWE bearing SRO family from *B. oleracea, B. carinata* and *B. juncea* and make a comparative assessment of their putative products using *in-silico* means.

METHODS

Obtaining sequences of orthologues

DNA was extracted from *B. carinata* (16195-NARC)*, B. juncea* (1664-NARC), *B. oleracea* (1739-NARC), *B. napus* (1679-NARC) and *A. thaliana* (Col-0) using the standard protocol (Doyle and Doyle, 1987). To these DNA, primers applied were designed using the PRIMER3 software (Untergrasser *et al.,* 2012). The primers were designed for three conserved regions of *A. thaliana* (TAIR Accession: AT1G32230.1) and *B. napus* (NCBI accession: BQ858405) orthologues of *rcd*1, i.e. upstream-WWE (seq1), WWE-PARP (seq2), PARP-RST (seq3), such that these fragments had overlaps at their 3' junctions. These designed primers (enlisted in Table 1) were applied to the extracted DNA. In these reactions, *B. napus* was used as the positive control. PCR was performed in triplicate and repeated at least thrice for the seq1, seq2 and seq3 of each individual species using the PCR recipe: 1X PCR buffer, 1.5 Mm MgCl$_2$, 0.2 Mm dNTPs, 0.5 μM of each primer and 1.5 U of Taq Polymerase. Initial denaturation was achieved at 94˚C for 4 mins, 35 cycles of denaturation at 94˚C for 50 seconds, annealing at 54˚C for 1 minute 30 seconds, extension at 72˚C for 1 min. while final extension for 10 mins at 72˚C.

The amplified fragments were purified using kit (MOLE-QULE-ON, New Zealand) and amplified using cloning vector pTZ57R/T of InsTAclone PCR Cloning Kit (Thermoscientific, USA) and IPTG/Xgal selection. Successfully amplified fragments were checked by colony PCR and their sequences were determined by sending them to a commercial laboratory (MOLEQULE-ON, New Zealand).

In-silico analyses of the orthologues

Reads designated as seq1, seq2 and seq3 for each individual species were aligned using the MULTALIN server (Corpet, 1988) to obtain full-length orthologue of the *rcd*1 for each individual species. Their reverse sequences were determined using the Reverse Compliment Finder (Stothard, 2000). 'Open Reading Frames' were determined using the ORF Finder (Rombel *et al.,* 2002), conceptually translated using the 'Expasy Translate Tool' (Gasteiger *et al.,* 2003), while their putative domains were identified using 'InterPro' (Finn *et al.,* 2016), similarity searches were performed using BLASTp against the refseq_proteins database (Altschul *et al.,* 1997), physical parameters were estimated using "PROTPARAM" (Gasteiger *et al.,* 2003), while its subcellular signals were defined by using WOLFPSORT (Horton *et al.,* 2007). Conceptually translated versions of the homologues were subjected to the determination of their folding capacities using 'Foldindex' (Prilusky *et al.,* 2005).

From the translated sequences, protein secondary structural elements were determined using CFSSP tool (Chou and Fasman, 1974), while QUARK and i-TASS-ER servers (Yang *et al.,* 2015; Xu and Zhang, 2012) were used for the 3D structure predictions. Full chain structures gave a low confidence score (data not shown where TM<0.5), hence individual domains (WWE, PARP and RST) were used for the structure predictions. Tertiary structure models, corresponding to the orthologues, were verified using verify3D (using residues/3D-ID Scores) (Eisenberg *et al.,* 1997). Probable binding sites within the structures were predicted using COACH metaserver to get an idea on putative ligands (Yang *et al.,* 2013) and the function prediction using *in-silico* models was performed using CO-FACTOR (Zhang *et al.,* 2017). For the assessment of transcription factor binding using CDS of homologues, PlantTFDB 4.0 was used (Jin *et al.,* 2017).

Table 1: Primers used in amplification of the *rcd*1 reads

Primer Name	Desired Region for Amplification	Primer Notation	Primer Sequence	Primer Length
BnU	Upstream and WWE encoding sequence of rcd1 in Brassicaceae	BnU-F	5'-CTTTCAGGTTATAGGTTTAGCATTG-3'	25
		BnU-R	5'-CATCAGTCTCATCCCATTTCG-3'	21
BnU-PARP	Upstream and PARP domain encoding sequence in Brassicaceae	BnU-PARP-F	5'-AGAAGACCGGAATTGCAAAG-3'	20
		BnU-PARP-R	5'-CTGTGCTTTATCACCACGAAGA-3'	22
BnPARP-RST	PARP and RST domains' encoding sequence in Brassicaceae	BnPARP-RST-F	5'-GAGCTTTTCCAGAAGCAGGTT-3'	21
		BnPARP-RST-R	5'-CACCTGCACCTTCCTCGT-3'	18

RESULTS

From the extracted DNA, the designed degenerate primers amplified products of nearly 800 bp for seq1, 1200 bp for seq2 and 1600 bp for seq3 are shown (Figure 1). The overlapping sequence was trimmed manually from the 5'-ends of the reads. After removing the sequence overlaps, gene sequences obtained were found to have coding sequences (CDS) of 1664 bp in case of *B. carinata,* 1954 bp in case of *B. juncea* and 2006 bp of *B. oleracea.* These sequences were submitted to NCBI and their issued accessions are MG570396, MG570397 and MG570398, respectively. Their corresponding translated products were 544 amino acids in *B. carinata*, 552aa in *B. juncea* and 562aa in *B. oleracea.* These *in-silico* translated products were named as BoRCD1, BcRCD1 and BjRCD1, corresponding to the initials of the *Brassica oleracea, Brassica carinata* and *Brassica juncea.* These proteins were found to bear the conserved domains of the RCD1/SRO1 type proteins, which are an N-terminal WWE (InterPro: IPR004170, ProSite: PS50918), central PARP (IPR012317, PFam: PF00644, PS51059) and C-terminal RST (IPR022003, PF12174). The presence of characteristic domains of the RCD1-type proteins in these translated products gave a clue to the presence of the versatile gene named *rcd*1 within these plants of agricultural importance. Physical parameters associated with these proteins suggest them to have an acidic nature and hence they are proposed to bear a net negative charge at the normal physiological pH, based on the *in-silico* analysis (Table 2). The instability indexes suggested them to be highly instable proteins within the plant cell environment (Table 2).

Within the subcellular spaces, the BoRCD1, BcRCD1 and BjRCD1 could be localized to several organelles, as indicated by the presence of signals for localization to/across cytoplasm, cytoskeleton, mitochondrion, plasma membrane; in addition to those for nuclear localization (Table 3). Out of the three reported NLS (Nuclear Localization Signal) signatures in *A. thaliana* RCD1 (AtRCD1) (by Bellex-bois, 2000), KKRKR (NLS1 of AtRCD1) and KKHR (NLS3) were found within the BcRCD1, BoRCD1 and BjRCD1. An important signal
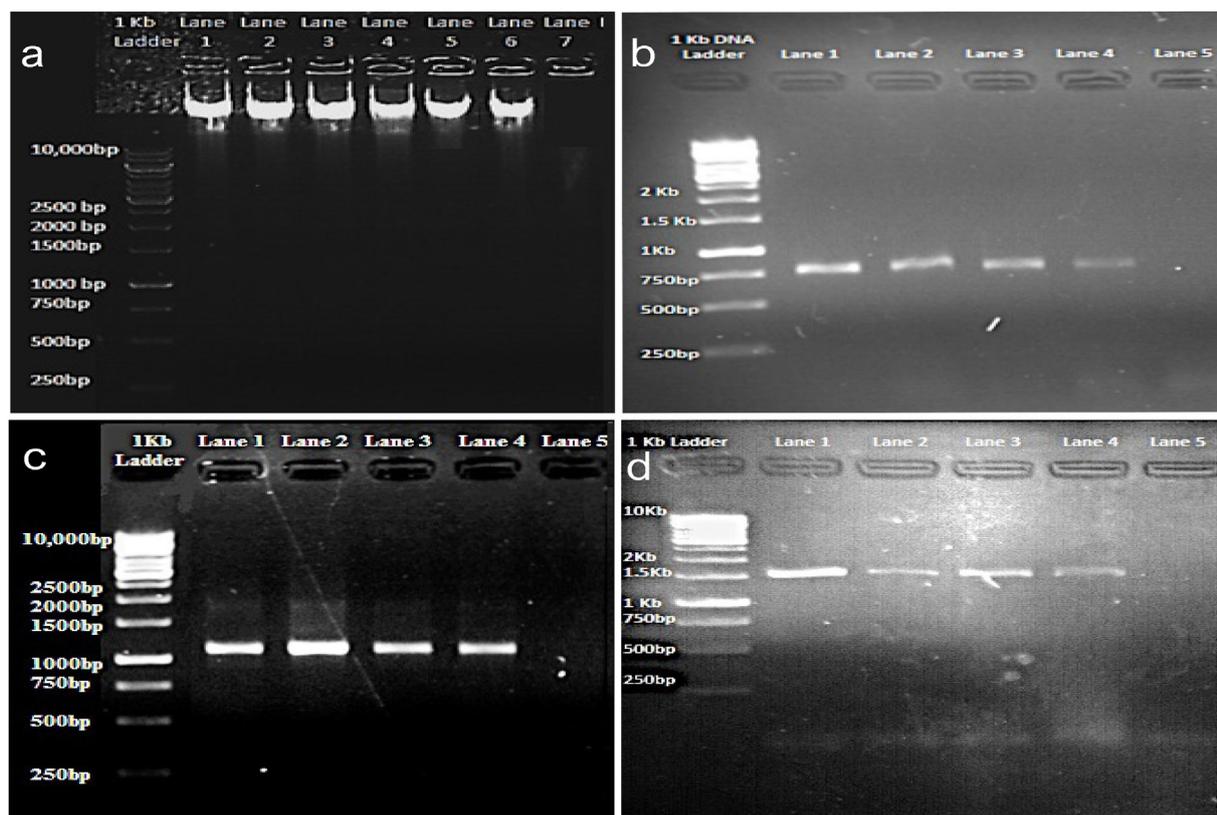

Figure 1: Gel images extracted DNA of B. carinata (Lane 1 and 2), B. juncea (Lane 3), B. oleracea (Lane 4), B. napus (Lane 5), A. thaliana (Lane 6), Blank (Lane 7). PCR Amplification of the overlapping putative rcd1 reads with Brassica oleracea in lane 1, Brassica carinata in lane 2, Brassica juncea in lane 3 and Brassica napus as positive control in lane 4 of each respective gel. b) Upstream region c) upstream-PARP encoding sequence d) PARP-RST encoding sequence

Table 2: Various physical parameters of the *Brassica* RCD1 proteins

RCD1 Homologue	Genbank Accession (Coding Sequence/Protein Sequence)	Number of amino acids	Molecular Weight (Daltons)	Isoelectric Point (pI)	Instability Index	Hydropathicity Grand Average
BcRCD1	MG570396.1/ AYF57917.1	544	62899.28	5.74	48.21	- 0.501
BoRCD1	MG570398/ AYF57919.1	562	62727.43	6.36	42.03	- 0.485
BjRCD1	MG570397/AYF57918.1	552	54743.08	5.14	40.31	- 0.415

of AtRCD1, designated as KRRR (NLS2), was found substituted for KRRKL in the inspected members of the genus *Brassica*, highlighting an R to K substitution in the signals of these *Brassica* genus plants.

Foldindex analysis revealed that these orthologues have 2 highly folded areas within the PARP region (residues 250 to 350) and a net total of 248-262 disordered residues (Supplementary data, Figure 1). Presence of disordered region supports the participation of these proteins in regulatory processes that affect a plant's survival. Prediction of transcription factor binding sites using p-value threshold set at p-value ≤ 0.5, resulted in prediction of 48 binding sites of 34 transcription factors from BoRCD1. Similar searches identified 28 sites from 26 transcription factors from BcRCD1 and 38 binding sites from 34 TFs from BjRCD1. Binding to an array of transcription factors is an attribute of a regulatory/hub proteins that take part in regulatory processes of a plant (Vandereyken *et al.*, 2018). The homologues bear motifs for interaction with transcription factors belonging to several families (TFs summarised in table 4), importantly with those belonging to AP2/ERF, as 25 such binding motifs have been identified for BoRCD1, 14 BcRCD1 and 12 for BjRCD1. The 3D structure remodelling using *in-silico* environment was performed for the whole proteins and this gave low scores owing to the presence of several stretches

of disordered residues amid domain forming stretches of amino acids (data not shown). This has also been indicated by the Foldindex analysis (Supplementary data, Figure 1). To get a better resolution, domain forming stretches of amino acids were subjected to *in-silico* structure determination using QUARK and i-TASSER servers. The structures of RST were determined with TM (Template Modelling) scores in the range of 0.7-0.85 (Table 5) and revealed the sole presence of alpha-helices being separated by turns and coils leading to an overall structure shown in figure 2. Similarly, the WWE forms an overall structure comprising a single large alpha-helix being separated by two b-sheets through some turns. This domain also shares structural homology to the pyridoxal kinases that have transferase activity *in-vivo*.

The structure-based molecular functional prediction for each of the *Brassica* PARP model was Gene Ontology (GO): 0003950 that outlines structures with an active PARP. The GO scores obtained (out of 1) were 0.90, 0.91 and 0.95 for BjRCD1, BoRCD1 and BcRCD1, respectively. Further studies were made using COFACTOR server, where PARP activity was predicted with a 69% for BcRCD1, 61% for BoRCD1 and 65% for BjRCD1 PARP. In further studies, we found these homologues to have good affinities for binding inhibitors like RGK (2-(4-Aminophenyl)-3,4-Dihydroquinazolin-4-One)

Table 3: Prediction of signals for subcellular localization

Brassica Homologue	Identifiers For Subcellular Localization				
	Nuclear	Cytoplasmic	Mitochondrial	Plasma Membranic	Cytoskeletonic
BcRCD1	At3g09840.1, At5g04940.1 At5g06460.1, At4g16420.1	EF2_BETVU, At1g77520.1 THS1_VITVI, At5g37170.1 EF2_CHLKE, THS2_VITVI	At4g13940.1	NRL3_ARATH	At2g35630.1
BoRCD1	At3g09840.1, At5g04940.1 At5g06460.1	EF2_BETVU, ACOC_ARATH At1g77520.1, THS2_VITVI At5g37170.1, THS1_VITVI SYK_LYCES	At4g13940.1	NRL3_ARATH	ACT7_ORYSA At2g35630.1
BjRCD1	At3g09840.1 At5g04940.1	PPC1_UROPA At1g76990.1 PPC2_UROPA	At4g13940.1	NRL3_ARATH	ACT1_ARATH, CT2_DAUCA ACT13_SOLTU, ACT1_SORBI ACT1_ORYSA

Table 4: Prediction of transcription factor binding of *Brassica* homologues

Brassica species	Transcription factors
B. carinata	ARF, E2F/DP, ERF, GATA,LBD, MYB, NAC,SBP, Trihelix
B.oleracea	ARF, Bzip,E2F/DP,ERF,GATA,LBD,MIKC_MADS,MYB,NAC,SBP,Trihelix, WKRY
B. juncea	ARF,ARR-B, B3,BBR-BPC,C2H2,E2F/DP,ERF,GATA, LBD,MIKC_MADS,MYB,NAC, Trihelix,SBP,WRKY

Table 5: Quality assessment results of the *in-silico* models

Brassica species	Template Modelling (Tm) Score of Models (Range: 0-1)			Verify3D (Percent Residues with 3D/ID Scores> 0.2)		
Domains	WWE	PARP	RST	WWE	PARP	RST
B. carinata	0.6148 ± 0.0764	0.85 ± 0.08	0.7967 ± 0.0764	98.4 %	86.5 %	93.8 %
B. oleracea	0.5947 ± 0.0764	0.69 ± 0.12	0.8074 ± 0.0764	97.8 %	93.6 %	90.2 %
B. juncea	0.6148 ± 0.0764	0.67 ± 0.12	0.8512 ± 0.0764	98.4 %	96.4 %	90.8 %

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and GAB (3-Aminobenzoic Acid) (Figure 3). The Confidence Score (CS) for BcRCD1 PARP with RGK was 0.77, BjRCD1 PARP with GAB was 0.65 and BoRCD1 PARP with GAB was 0.66. These are the inhibitors of PARP activity. The key residues comprising this binding site are predicted to be LP-HLT-FS-Y-N. The PARP structures of newly found orthologues seem to be closely related to the AtRCD1 PARP, as determined using superimposition procedures (Figure 3).

TM-Align has identified the crystal structure of the RCD1 of *A. thaliana* PARP (with PDB id: 5ngoA) to be closely related to that of *B. carinata* PARP, having an RMSD (Root Mean Square Deviation) of 0.82 over a coverage of 0.986. The TM scores obtained were 0.958/1 for BcRCD1, 0.794/1 for BoRCD1 and 0.779/1 for BjRCD1. The superimposition has been shown in Figure 4, indicating these proteins to be closely related in structure and function to the AtRCD1.
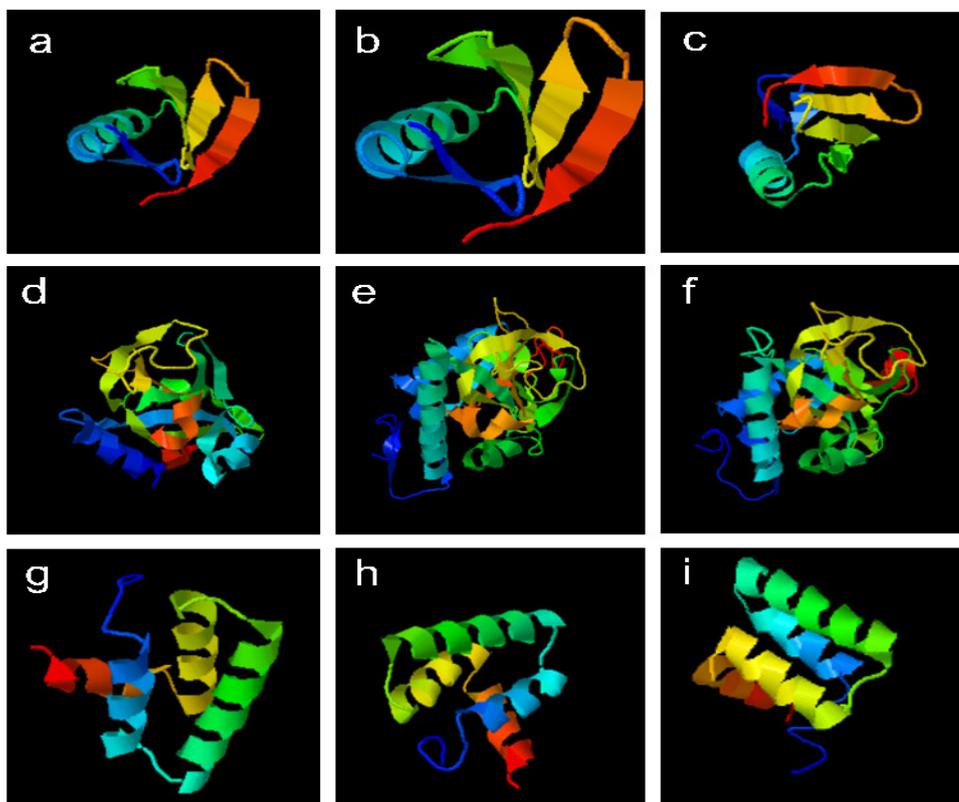

Figure 2: Structures of Brassica homologues' domains: a) BC WWE b) BJ WWE c) BO WWE d) BC PARP e) BJ PARP f) BO PARP g) BC RST h) BJ RST i) BO RST
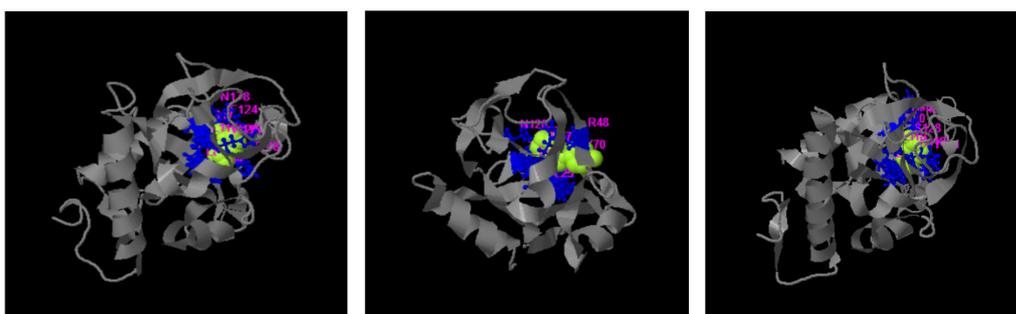

Figure 3: Ligand Binding Prediction of Brassica PARPs a) BC PARP with RGK b) BJ PARP with GAB c) BO PARP with GAB

Figure 4: Superimposition of the PARP models of newly found Brassica homologues of RCD1 with the crystal structure of A. thaliana PARP (5ngoA) using TM-Align, where ribbon indicates Brassica PARP and discreet line indicates A. thaliana PARP. a) B. carinata b) B. juncea c) B. oleracea

Some greater contribution towards the functions exhibited by these homologues can come from the PARP domain if it's catalytically active, as indicated in the consensus prediction of the function for PARP (Figure 5); indicating these proteins to be involved in a myriad of developmental and stress-related pathways. Some pathways predicted with a high confidence (>0.9) are response to stress (especially salt stress). Hence, the findings suggest the found genes to encode proteins homologous to the RCD1 of AtRCD1 and highlight a prospect of being a potential candidate for the development of resistant cultivars using breeding and/or biotechnological approaches.

DISCUSSION

Bringing genes that regulate multiple stresses on forefront and studying their properties could play some role in the biotechnological approaches that aim at making crops resilient to the environmental upsets (Meyer and Purugannan, 2013). As part of our current finding, the versatile RCD1 of *Arabidopsis thaliana* has its orthologous relatives within three cultivated varieties of the *Brassica* genus (*B. carinata, B. juncea, B. oleracea*). The amplification of the orthologues from the family-related species; i.e. *Brassica carinata, B. juncea* and *B. oleracea*, using primers designed on conserved genomic regions from *Brassica napus* and *Arabidopsis thaliana* orthologues of the *rcd*1; demonstrate that these
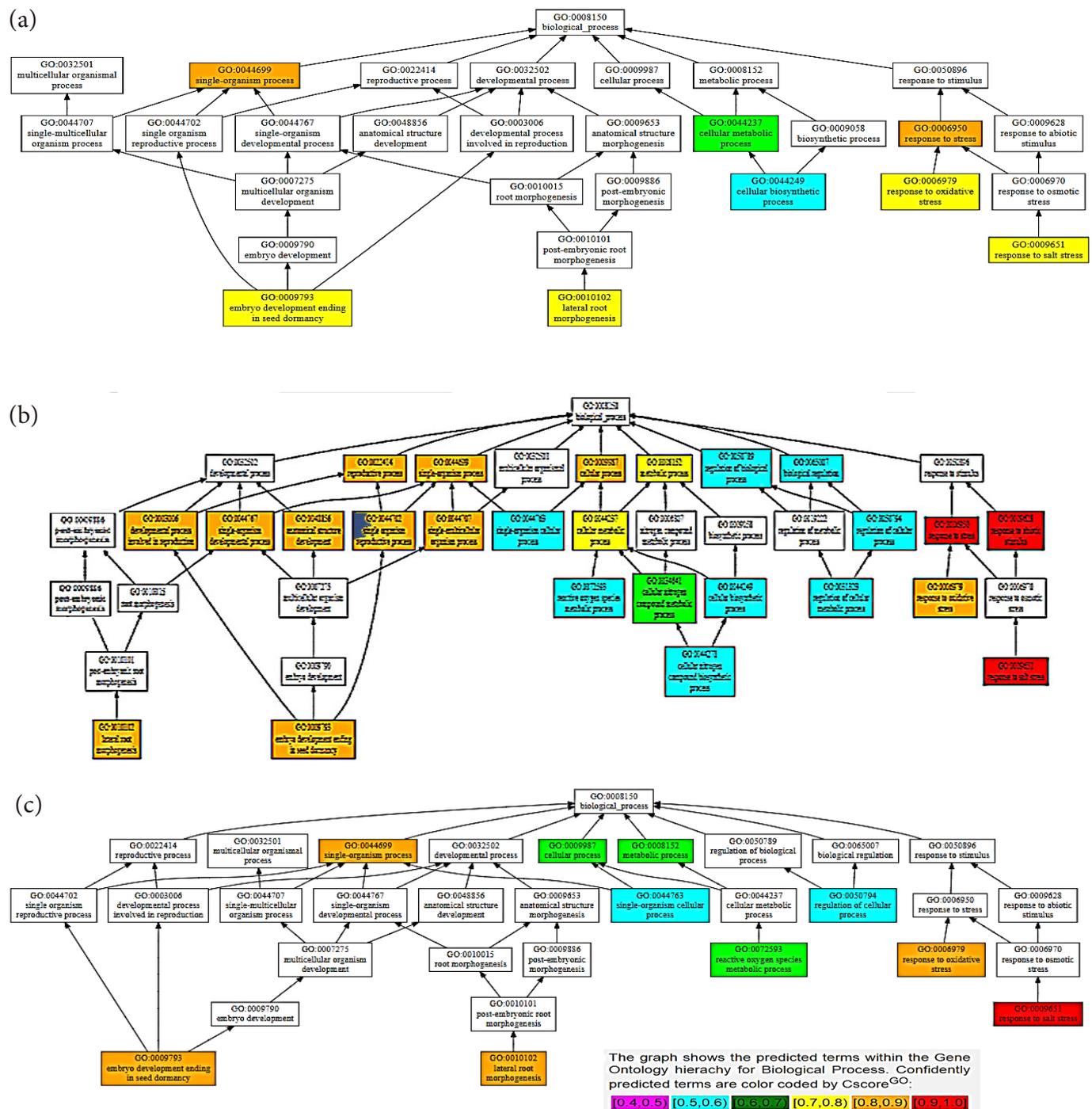

Figure 5: Consensus Prediction of Biological Function of the domains of BcRCD1, BoRCD1 and BjRCD1 a) WWE b) PARP c) RST

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orthologues share some degree of sequence conservation across these closely related plants. This feature has also been demonstrated in a previous research in which the members of various plant families have been found forming phylogenetic clades being supported through high bootstrap values (Siddiqua *et al.*, 2016).

The analysis of the putative protein products encoded by these genes, show that they bear complete WWE domain alongside PARP and RST domains. The WWE domain characteristically exists within the active members RCD1 and SRO1 of *A. thaliana*, both of which demonstrate their functions through genetic redundancies (Jaspers *et al.*, 2009, Teotia and Lamb, 2009). Aside the WWE domain, at N-terminal end of the protein, three signals for nuclear localization, KKRKR (NLS1), KRRR (NLS2) and KKHR (NLS3), have been reported in the AtRCD1 (Belles-Boix *et al.*, 2000). Two of these signals; NLS1 and NLS2, are reported to be essential for the nuclear localization of AtRCD1 under unstressed situations. While upon exposure to stress, AtRCD1 has been shown to be found within cytoplasm as well as in nucleus (Katiyar-Agarwal *et al.*, 2006). These proteins also have instability indices of 40 or above and such protein are instable *in-vivo* (Guruprasad *et al.,* 1990). The signals obtained within the conceptually translated Brassica homologues show their import-export into several plant cellular compartments. The absence of the NLS2, which has been shown to be of importance for the nuclear localization of the AtRCD1 by Katiyar-Agarwal and coworkers, indicates some reduced localization of this protein in nucleus in comparison to the AtRCD1. Moreover, the presence of signatures for mitochondrial and chloroplastic import indicate chances of retrograde signalling involving RCD1. Signals for transport to plasma membrane have also been identified within these orthologues of AtRCD1, where SOS1 resides that has previously been indicated to interact with AtRCD1 (Katiyar-Agarwal *et al.*, 2006). The Arabidopsis RCD1 has been shown to regulate signaling from chloroplasts and mitochondria to establish transcriptional control over the regulatory processes in these organelles (Shapiguzov *et al.*, 2019).

Some idea on the functional characteristics of these orthologues was drawn using the *in-silico* structural data. The structure-based function prediction brings important highlights on the *in-vivo* characteristics of a protein (Wilkins *et al.*, 2012). Such approach has revealed these newly found RCD1 homologues to be involved in oxidative and salinity responses among stress-responsive pipeline. This finding corroborates with the previous findings in AtRCD1 that the protein interacts with SOS1 tails and controls set of genes that respond to salt and ROS generated stress signals (Belles-boix *et al.*, 2000; Katiyar-Agarwal *et al.,* 2006) while, among the developmental pathways, the consensus prediction has been of lateral root morphogenesis and embryonic development ending in seed-dormancy. The expression of AtRCD1 in root-tip has been observed using GUS-tagged promotors' expression (Katiyar-Agarwal *et al.,* 2006). Some similar results were obtained in case of RCD1/SRO1 double mutants in *A. thaliana* when lesser number of seeds sprouted and developed into plants (Jaspers *et al.,*

2009) that gives some clues that the *Brassica* homologues share functional similarity to AtRCD1. Importantly, this structure-based function prediction has indicated PARP activity, marginally, for these homologues that share some degree of structural analogy to the *A. thaliana* PARP. On the other hand, this *in-silico* finding has also identified PARP domains of BcRCD1, BjRCD1 and BoRCD1 in binding the inhibitors GAB and RGK. This binding to inhibitors could also lead to an inactive PARP. Such suggestion has also been previously made about AtRCD1 (Jaspers *et al.,* 2010). PARPs regulate highly important cell functions that include gene expression regulation, programmed cell death and DNA damage response, to name a few (Vainonen *et al.*, 2016). Hence, the activity displayed by PARP could modulate the overall function displayed by the RCD1 homologues and could be the potential target of crop-improvement strategies. This is of importance as it has currently been suggested that versatile functions exhibited by AtRCD1 has no involvement of PARP activity (Wirthmueller *et al.*, 2017). On the other hand, it has been demonstrated that the presence of an active PARP within the RCD1 could drastically improve the stress tolerance potential of the reservoir plant (Liu *et al.,* 2014). Hence, owing to the absence of catalytic binding triad of H-Y-E within the binding site and close similarity to the PARP of AtRCD1, it is being suggested that the PARP might not be catalytically active within these orthologues. Based on structure-based function prediction of all domains (Figure 4), we can conclude that these homologues have high chances of being involved in responses to salinity and oxidative stresses among stress-responsive pathways and in embryonic seed development that ends in dormancy and lateral root morphogenesis among the developmental pathways.

The RST domains have important role in binding transcription factors (Katiyar-Agarwal *et al.*, 2006; Jaspers *et al.,* 2009; You *et al.,* 2014). In *A. thaliana*, at least 21 transcription factors have been shown to interact through the RST domains to TFs like DREB2A (of AP2/ERF class) that regulate multiple stresses like drought and heat (Belles-Boix *et al.,* 2000; Jaspers *et al.,* 2009). The TFs predicted to have binding affinities for *Brassica* homologues fall under diverse classes like MYB, GATA, NAC, that play diverse roles of stress regulation (Reviewed in Lindemoss *et al.,* 2013). Of particular importance are the enrichment of motifs within newly found orthologues for the AP2/ERF class of TFs, as these TFs participate in wide variety of stress and developmental responses and are current targets for crop improvement perspectives (Phukan *et al.,* 2017). In the *Brassica* orthologues of AtRCD1, the RST domain was found comprising a structure that was solely represented by alpha helices, and this has also been shown to be the case with *A. thaliana* RST whose structural constrains have been determined using NMR (Nucleic Magnetic Resonance) (Tossavainen *et al.,* 2017). Hence, the overall analysis suggests that there exists some degree of conservation with respect to sequence, structure and function across RCD1 orthologues within the family of Brassicaceae.

REFERENCES

Ahlfors R., Lång S., Overmyer K., Jaspers P., Brosché M., Tauriainen A., Kollist H., Tuominen H., Belles-Boix E., Piippo M., Inzé D. (2004). *Arabidopsis* Radical-Induced Cell Death1 belongs to the WWE protein–protein interaction domain protein family and modulates abscisic acid, ethylene, and methyl jasmonate responses. *The Plant Cell Online*, 16: 1925-1937.

Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z., Miller W., Lipman D.J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25: 3389-3402.

Anjum S., Raza S., Azhar A., Qamarunnisa S. (2015). Bnsro1: A new homologue of *Arabidopsis thaliana* rcd1 from *Brassica napus*. *Biologia,* 70: 588-598.

Aravind L. (2001). The WWE domain: a common interaction module in protein ubiquitination and ADP ribosylation. *Trends Biochem. Sci,* 26:273–5.

Belles-Boix E., Babiychuk E., Van Montagu M., Inze D., Kushnir S. (2000). CEO1, a new protein from *Arabidopsis thaliana*, protects yeast against oxidative damage. *FEBS Lett*, 482:19–24.

Brosché M., Blomster T., Salojärvi J., Cui F., Sipari N., Leppälä J., Lamminmäki A., Tomai G., Narayanasamy S., Reddy R.A., Keinänen M. (2014). Transcriptomics and functional genomics of ROS-induced cell death regulation by Radical-Induced Cell Death1. *PLoS Genet*, 10: e1004112.

Chou P.Y., Fasman G.D. (1974). Prediction of protein conformation. *Biochemistry*, 13:222-245.

Corpet F. (1988). Multiple sequence alignment with hierarchical clustering. *Nucleic Acids Research* 16: 10881-10890.

Darwin C., Bynum W.F. (2009). The origin of species by means of natural selection: or, the preservation of favoured races in the struggle for life. *Al Burt,* 441-764.

Doyle J.J., Doyle J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.,* 19: 11-15.

Eisenberg D., Lüthy R., Bowie J.U. (1997). VerifyFY3D: Assessment of protein models with three-dimensional profiles. *Methods in Enzymology*, 277: 396-404.

Finn R.D., Attwood T.K., Babbitt P.C., Bateman A., Bork P., Bridge A.J., Chang H.Y., Dosztányi Z., El-Gebali S., Fraser M., Gough J. (2016). InterPro in 2017-beyond protein family and domain annotations. *Nucleic Acids Research,* 45:190-199.

Gasteiger E., Gattiker A., Hoogland C., Ivanyi I., Appel R.D., Bairoch A. (2003). ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res,* 31: 3784-3788.

Geer L.Y., Domrachev M., Lipman D.J., Bryant S.H. (2002). CDART: protein homology by domain architecture. *Genome Res.,* 12:1619-1623.

Goodstein D.M., Shu S., Howson R., Neupane R., Hayes R.D., Fazo J., Mitros T., Dirks W., Hellsten U., Putnam N., Rokhsar D.S. (2011). Phytozome: a comparative platform for green plant genomics. *Nucleic Acids Research*, 40:1178-1186.

Guruprasad K., Reddy B.B., Pandit M.W. (1990). Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting *in vivo* stability of a protein from its primary sequence. Protein Engineering, *Design and Selection,* 4:155-161.

Horton P., Park K.J., Obayashi T., Fujita N., Harada H., Adams-Collier C.J., Nakai K. (2007). WoLF PSORT: protein localization predictor. *Nucleic Acids Research*, 35: 585-587.

Jaspers P., Blomster T., Brosche M., Salojärvi J., Ahlfors R., Vainonen J.P., Reddy R.A., Immink R., Angenent G., Turck F., Overmyer K. (2009). unequally redundant RCD1 and SRO1 mediate stress and developmental responses and interact with transcription factors. *Plant J. Cell Mol. Biol.,* 60: 268–279.

Jaspers P., Overmyer K., Wrzaczek M., Vainonen J.P., Blomster T., Salojärvi J., Reddy R.A., Kangasjärvi J. (2010a). The RST and PARP-like domain containing SRO protein family: analysis of protein structure, function and conservation in land plants. *BMC Genomics,* 11:170.

Jaspers P., Brosché M., Overmyer K., and Kangasjärvi J. (2010b). The transcription factor interacting protein RCD1 contains a novel conserved domain. *Plant Signal. Behav.,* 5:78–80.

Jin J.P., Tian F., Yang D.C., Meng Y.Q., Kong L., Luo J.C., Gao G. (2017). PlantTFDB 4.0: toward a central hub for transcription factors and regulatory interactions in plants. *Nucleic Acids Research*, 45:1040-1045.

Katiyar-Agarwal S., Zhu J., Kim K., Agarwal M., Fu X., Huang A., Zhu J.K. (2006). The plasma membrane Na^+/H^+ antiporter SOS1 interacts with RCD1 and functions in oxidative stress tolerance in *Arabidopsis. Proc. Natl. Acad. Sci.,* 103:18816–18821.

Lindemose S., O'Shea C., Jensen M.K., Skriver K. (2013). Structure, function and networks of transcription factors involved in abiotic stress responses. *International Journal of Molecular Sciences*, 14: 5842-5878.

Liu S., Liu S., Wang M., Wei T., Meng C., Wang M., Xia G. (2014). A wheat SIMILAR TO RCD-ONE gene enhances seedling growth and abiotic stress resistance by modulating redox homeostasis and maintaining genomic integrity. *The Plant Cell,* 26: 164-180.

Marchler-Bauer A., Bo Y., Han L., He J., Lanczycki C.J., Lu S., Chitsaz F., Derbyshire M.K., Geer R.C., Gonzales N.R., Gwadz M. (2017). CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. *Nucleic Acids Research*, 45:200-203.

Meyer R.S., Purugganan M.D. (2013). Evolution of crop species: genetics of domestication and diversification. *Nature Reviews Genetics*, 14:840-852.

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Mur L.A., Kenton P., Atzorn R., Miersch O., Wasternack C. (2006). The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. *Plant Physiology,* 140: 249-262.

Overmyer K., Tuominen H., Kettunen R., Betz C., Langebartels C., Sandermann Jr H., Kangasjarvi J. (2000). Ozone-sensitive *Arabidopsis* rcd1 mutant reveals opposite roles for ethylene and jasmonate signalling pathways in regulating superoxide-dependent cell death. *The Plant Cell,* 12: 1849–1862.

Parkin I.A., Koh C., Tang H., Robinson S.J., Kagale S., Clarke W.E., Town C.D., Nixon J., Krishnakumar V., Bidwell S.L., Denoeud F. (2014). Transcriptome and methylome profiling reveals relics of genome dominance in the mesopolyploid B*rassica olerace*a. *Genome Biology,* 15:77.

Phukan U.J., Jeena G.S., Tripathi V., Shukla R.K. (2017). Regulation of Apetala2/Ethylene response factors in plants. *Frontiers in Plant Science,* 8.

Prakash C.S. (2001). The genetically modified crop debate in the context of agricultural evolution. *Plant Physiology*, 126: 8-15.

Prilusky J., Felder C.E., Zeev-Ben-Mordehai T., Rydberg E.H., Man O., Beckmann J.S., Silman I., Sussman J.L. (2005). FoldIndex©: a simple tool to predict whether a given protein sequence is intrinsically unfolded. *Bioinformatics,* 21: 3435-3438.

Rejeb I.B., Pastor V., Mauch-Mani B. (2014). Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms. *Plants* 3:458-475.

Rombel I.T., Sykes K.F., Rayner S., Johnston S.A. (2002). ORF-Finder: a vector for high-throughput gene identification. *Gene,* 282: 33-41.

Siddiqua B., Qamarunnisa S., Azhar A. (2016). RCD1 homologues and their constituent WWE domain in plants: analysis of conservation through phylogeny methods. *Biologia,* 71: 642-650.

Stothard P. (2000). The sequence manipulation suite: JavaScript programs for analyzing and formatting protein and DNA sequences. *BioTechnique*s, 28:1102-1104.

Teotia S., Lamb R.S. (2009). The paralogous genes Radical-Induced Cell Death1 and Similar to RCD One1 have partially redundant functions during *Arabidopsis* development. *Plant Physiology*, 151:180-198.

Tossavainen H., Hellman M., Vainonen J.P., Kangasjärvi J., Permi P. (2017). 1H, 13C and 15N NMR chemical shift assignments of *A. thaliana* RCD1 RST. *Biomolecular NMR Assignments*, 1-4.

Vainonen J., Shapiguzov A., Vaattovaara A., Kangasjärvi J. (2016). Plant PARPs, PARGs and PARP-like Proteins. *Current Protein and Peptide Science*, 17: 713-723.

Vandereyken K., Van Leene J., De Coninck B., Cammue B. (2018). Hub protein controversy: taking a closer look at plant stress response hubs. *Frontiers in Plant Science*, 9, 694.

Wilkins A.D., Bachman B.J., Erdin S., Lichtarge O. (2012). The use of evolutionary patterns in protein annotation. *Curr. Opin. Struc. Biol.,* 22: 316–325.

Wirthmueller L., Asai S., Rallapalli G., Sklenar J., Fabro G., Kim D.S., Lintermann R., Jaspers P., Wrzaczek M., Kangasjärvi J., MacLean D. (2017). An oomycete effector protein induces shade avoidance in *Arabidopsis* and attenuates salicylate signaling by binding to host proteins of the Radical-Induced Cell Death1 family. *bioRxiv*, 137844.

Xu D., Zhang Y. (2012). *Ab initio* protein structure assembly using continuous structure fragments and optimized knowledge-based force field. *Proteins*, 80:1715-1735.

Yang J., Roy A., Zhang Y. (2013). Protein-ligand binding site recognition using complementary binding-specific substructure comparison and sequence profile alignment. *Bioinformatics,* 29:2588-2595.

Yang J., Yan R., Roy A., Xu D., Poisson J., Zhang Y. (2015). The I-Tasser Suite: protein structure and function prediction. *Nature Methods*, 12: 7-8.

You J., Zong W., Du H., Hu H., Xiong L. (2014). A special member of the rice SRO family, OsSRO1c, mediates responses to multiple abiotic stresses through interaction with various transcription factors. *Plant Molecular Biology,* 84:693–705.

Zhang C., Freddolino P.L., Zhang Y. (2017). COFACTOR: improved protein function prediction by combining structure, sequence and protein–protein interaction information. *Nucleic Acids Research*, 45:291-299.