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In Silico Elucidating the Common Gene-Regulatory-Network(S) Of Accelerated Cell Death (Acd11) from *Arabidopsis Thaliana* and Its Orthologs

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ABSTRACT

The Arabidopsis accelerated cell death 11 (acd11) is a genetic model for studying immune response activation and localized cellular suicide that prevent pathogen spread during plant infection. In this research article we predicted the common gene-regulatory-network of accelerated cell death (acd11) from Arabidopsis thaliana and its orthologs with the help of well-known bioinformatics tools. The most common orthologs were extracted from Phytzome and PLAZA on the basis of protein sequence similarity using BLASTp. Developmental expression analysis from Genevestigator concluded the acd11 is highly expressed at last developmental stage and co-expressed with Autophagy related and Disease resistant genes. PPI from STRING network confirm that its interacting partners are EDS1 and LAZ1 both of which play an important role in disease resistant and programmed cell death respectively. Protein disorder from DisEMBL confirm its diverse functioning properties and non-stable structure. Ligand binding study from RaptorX predicted that residues in the Active site pocket Asparagine and Histidine.

Keywords: *Arabidopsis accelerated cell death 11, acd11, BLASTp*

INTRODUCTION

Due to the hypersensitive (HR) response in plants, which causes PCD, the plant host resistance (R) protein identifies pathogen avirulence component. In the model plant Arabidopsis, recessive acd11 is a lesion mimic mutant that activates HR-like PCD in the absence of pathogen infection. Before the plant flowers, Acd11 starts to degrade the chlorophyll in the two-leaf stage, consuming the entire plant (Brodersen *et al*., 2002). Sphingolipid transfer proteins that help sphingosine transfer across membranes in vitro

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are homologous to ACD11 (Brodersen *et al*., 2002). ACD11 has similarity to GLTP, a protein that facilitates the transfer of glycoshingolipids. Although mammalian GLTP is structurally and biophysically well described, its biological function is unknown (Brown and Mattjus, 2007). The positively charged triad's clustered Lys/Arg residue in ACD11, which is best positioned to bind phosphate, explains why the protein is unable to bind sugar head groups or transfer glycolipids (Peterson *et al*., 2008).

ACD11 in yeast forms interactions with PRA7 and PRA8, while VAP27-1 has a stronger interaction with an ACD11 homolog rather than the ACD11 protein itself. While its interactors have varying degrees of membrane association, ACD11 is mostly a cytosolic protein. ACD11, in conjunction with the proteins PRA and VAP, may control membrane trafficking (Peterson *et al*., 2009).

To regulate programmed cell death (PCD), it is essential to maintain a dynamic balance between Cer and C1P as per the studies by Chen *et al.* (2009), Pata *et al.* (2010), and Berkey *et al.* (2012). The deficiency of *acd11* results in altered C1P levels and increased phyto-cer levels, indicating a functional connection between *acd11* expression and sphingolipid metabolic regulation in plants. Despite the low sequence similarity among ACD11 and other GLTP homologs, such as human CPTP, Simanshu *et al*. (2014) have demonstrated the existence of conserved structural similarity between Arabidopsis ACD11, human GLTP, and human CPTP.

OBJECTIVES

1. Identification and extraction of orthologs of ACD11 from *Arabidopsis thaliana* among plant species.

2. Screen for common interacting protein with ACD11 and its orhtologs to analyze and deduce the gene regulatory network of ACD11.

3. Prediction of 3D structure and interacting residue of proteins by determining its interface residue, intrinsic protein disorder.

LITERATURE REVIEW

Simanshu *et al.* (2014) have demonstrated that ACD11, encoded by the *acd11* gene, is a lipid transfer protein that promotes intermembrane transfer of sphingosine and sphingomyelin but not Cer or glycolceremides. *Acd11* is a lesion mimic mutant in the model plant *Arabidopsis*, and in its recessive form, it triggers HR-like PCD even without pathogen infection. At the two-leaf stage, *Acd11* induces chlorophyll degradation, which eventually engulfs the entire plant before flowering. Despite being similar to three known sphingolipid transfer proteins, ACD11 has been demonstrated to aid the transfer of sphingosine between membranes in laboratory experiments.

The discovery of lesion mimic mutants (LMM), such as the hypersensitive response (HR), which is associated with pathogen resistance, has proven to be a useful tool in studying programmed cell death (PCD) pathways in plants (Lorrain *et al.*, 2003).

The incompatibility reaction, a cell death response that takes place when cells of different genotypes fuse in filamentous fungi, is governed by a group of loci called *het* loci (heterokaryon incompatibility loci). In *Podospora anserina*, the genes upregulated during this cell death response are involved in a process similar to autophagy, and ACD11 homolog, called HET-C protein, is implicated in vegetative incompatibility reaction in the fungus (Pinan-Lucarre *et al.,* 2003).

Plants rely on programmed cell death (PCD) as an essential response mechanism to various internal and environmental signals to achieve proper development and survival. Unlike animal apoptosis, PCD in plants is characterized by the absence of engulfment by neighboring cells. PCD occurs in plants during development, virulent infections, and the hypersensitive response (HR) brought about by avirulent stress effectors, as noted by Lam and colleagues in 2004.

The plant surveillance system has evolved to recognise pathogen-secreted chemicals in order to initiate a defense response when plant pcd coexists with disease resistance, an occurrence known as the hypersensitive response. These released compounds function as virulence factors in plants lacking genetic disease resistance, acting through mostly unidentified methods. If the function of ACD11 is lost, the result is a lethal condition. The recessive homozygous mutant of *acd11* grow normally until they develop 2-4 leaves. After that, they exhibit accelerated cell death similar to that observed in lesion mimic mutant, which is a plant with spontaneous lesions on the leaves (Greenberg *et al*., 2004).

Broderson *et al.* (2005) have reported that salicylic acid (SA) levels increase in response to infections that induce programmed cell death (PCD), and salicylate hydroxylase, which is encoded by the bacterial nahG gene, can prevent PCD development. SA is believed to play a role in the initiation of PCD linked to pathogen defense responses. PCD in acd11 is reliant on the plant hormone SA and can be hindered by expressing a bacterial SA hydroxylase (nahG) or through mutation in PAD4 and EDS1.

Airene et al. (2006), Brown and Mattjus (2007), and Peterson et al. (2008) have suggested that ACD11 has a GLTP fold and acts as a glycolipid transfer protein, based on its predicted structural homology model. Meanwhile, Hofius et al. (2007) reported that programmed cell death (PCD) during the hypersensitive response (HR) in plants is triggered by recognition of pathogen avirulence factors by the plant host resistance (R) protein.

According to research by various authors, including Brown and Mattjus (2007), GLTP and FAPP2 are homologs of ACD11 that are involved in the transfer of glycosphingolipids between membranes, although the exact role of GLTP remains

unknown. ACD11 and ACD5 are involved in regulating the balance of Cer and C1P levels, which control PCD related to HR. Additionally, ACD11 may play a role in regulating membrane trafficking through interactions with PRA and VAP proteins, despite its inability to transfer glycolipids due to the Lys/Arg residue cluster. Studies also suggest that ACD11 expression is involved in regulating sphingolipid metabolism in plants, and its deficiency can lead to inappropriate HR activation by LAZ5 (Chen *et al*., 2009; Pata *et al.,* 2010; Berkey *et al*., 2012; Peterson *et al.,* 2008, 2009; Wang *et al.,* 2008; Palma *et al*., 2010).

LAZ1, a DFU300 transmembrane protein, serves as a regulator of PCD associated with HR and suppresses cell death in acd11 and certain types of HR cell death (Malinovsky et al., 2010). Although ACD11 and other GLTP homologs, including human CPTP, have low sequence homology, crystallographic data has established their conserved structural homology (Simanshu et al., 2014).

RESEARCH METHODOLOGY

The research methodology of this research article is described in the following subsections.

Orthologous genes of ACD11 that is identifie and selected on the basis of protein sequence similarity from PLAZA, PHYTOZOME (Goodstein *et al*., 2012) and NCBI Databases.

Protein sequences of the orthologous genes will be retrieved from Phytozome for further analysis.

Gene regulatory network will be predicted on the basis of tissue or conditional specific co-expressional behavior of genes *acd11,* using expression browser Databases such as eFP and tools like Planex (Yim*et al*., 2013), PlaNet (Mutwil *et al*., 2011) and Genevestigator (Zimmerman *et al*., 2004),

PPI network of ACD11 and orthologous genes will be retrieved from STRING (Szklarczyk *et al*., 2011) to predict common interacting protein. 3D structure of all interacting protein will be predicted from PHYRE2 and HHPred.

DisEMBL is a useful method for predicting protein structure disorder, and RaptorX is a web-based tool used for determining the functional role of proteins by generating reliable three-dimensional atomic models of proteins. These tools are important for target selection and constructing biochemical studies, particularly in genomics and structural biology projects. In addition, protein sequences and structures can be utilized to forecast residues that participate in ligand binding sites (LBS) (Linding and Jenssen *et al.,* 2003; Wang and Li *et al*., 2016).

RESULT AND DISCUSSIONS

Identification of orthologous for the reference Protein ACD11

For the reference gene ACD 11 of Arabdosis Thaliana were retrieved and identified through MSA BLAST tool form NCBI and Phytozome on the basis of protein structure similarity. From the following retrieved data of orthologues from databases, further selection was made on the basis of their availability for protein interaction and co-expression analysis.

Table 1. Representing ACD 11 (AT2G34690) orthologous genes with GeneInfo Identifiers from different plant species Data obtained from PLAZ and Phytozome BLASTp search.

GENE ID PLAZA	**GENE ID PHYTOZOME**	**ORGANISM**	**PROTEIN LEGTH**	**E-VALUE**	**BIT SCORE**	**% IDENTITY**
CRU_004G15840	Carubv10023844m.g	*C.rubella*	207	4e-140	400	92.75
TC0002G01160	Thecc1EG006094	*T. cacao*	207	5e-108	316	72.46
GR08G04690	Gorai.008G046900	*G. raimondii*	207	2e-106	312	71.98
MD10G020560	MDP0000162581	*M.domestica*	207	2e-103	304	71.01
RC28617G00100	**28617.t000010**	***R. communis***	**214**	**2e-103**	**305**	**69.16**
PPE_004G08510	Prupe.4G089300	*Prunus persica*	201	5e-102	302	70.65
FV3G15140		*Fragaria*	201	1e-99	295	71.64

	gene28951-v1.0-hybrid	*vesca*				
SL02G070250	**Solyc02g070250.2**	***S.lycopersicum***	**205**	**1e-96**	**287**	**68.78**
ZM01G55250	**GRMZM2G058872**	***Zea mays***	**209**	**1e-96**	**289**	**67.46**
OS03G57140	**LOC_Os03g57140**	***Oryza Sativa j***	**209**	**9e-93**	**277**	**61.72**
CS00010G00480	1g027852m.g	*Citrus cinensis*	171	9e-87	262	74.27
MT2G029520	**Medtr2g030530**	***M.truncata***	**209**	**5e-86**	**260**	**61.24**
PT06G05180	**Potri.006G051800**	***P.trichocarpa***	**171**	**5e-83**	**253**	**71.35**
GLYMA15G13470	**GM.15G13470**	***Glycine max***	**209**	**7e-99**	**293**	**70.05**

The plant species selected for further study have almost the same number of Amino acids in polypepetide chain with variations between 214 aa to 171 aa. The selection of species cover both dicots (*S.lycopersicum, M.truncata, P.trichocarpa, R.cummunis*) and monocots (*Zea mays and Oryza sativa*), for predication their protein structure, function and interactions to validate the reference gene and protein regulatory network analysis.

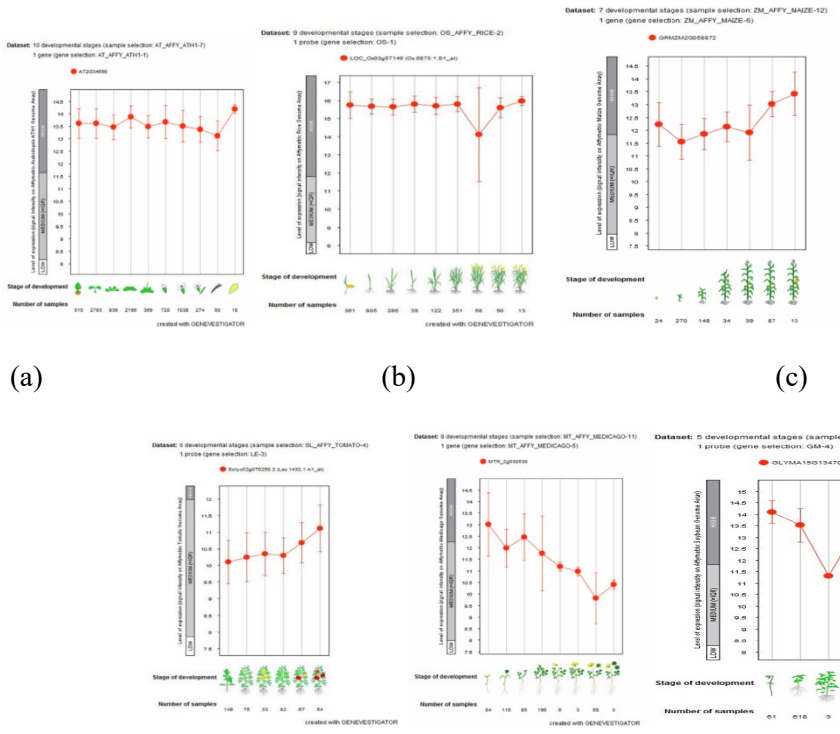
Extraction of protein sequences of ACD11 orthologs

Protein sequences in FASTA format of the orthologues gene were retrieved from the Phytozome database for further analysis of protein structure prediction, ligand binding, protein interaction, protein disorder all based on the sequence of proteins.

Predicting developmental stages co-expression of ACD11 and its orthologues

The expression of genes was examined during plant development and at the anatomical level by accessing the Affymetrix array database using the Genevestigator response viewer. Microarray data from the Arabidopsis Gene Chip platform was obtained and analyzed by querying gene member IDs. The database contains microarray data from only the wild-type background, and the expression level was assessed in the late stages of plant development, including senescence. ACD11 loss-of-function mutants exhibit accelerated cell death similar to that observed in lesion mimic mutants, and the protein may play a functional role during plant maturation and senescence (Greenberg et al., 2004).

The co-expressed gene were retrieved form the data for the refence gene as well as of their orthologues.



(a) (b) (c)

(d) (e) (f)

Figure 1 Developmental stage-dependent expression analysis of the reference gene and its orthologues, in most cases the genes expression is at highest level in the last

developmental stages of plant. (a) Arabidopsis thaliana (b) Oryza staiva (c) Zea mayz (d) Lycopersicum esculentum (e) Medicago truncatula (f) Glycine max

Co-expression were observed under the stress conditions (Perturbations) by default with a 50 limit of expressed genes. Positive correlation of the expressed genes under the same conditions were analyzed Pearson correlation through Genevistiagtor affymytrix biochips. All genes of the orthologues were put to the same conditions to confirm validation of the co-expressed genes with ACD11 of Arabidopsis thaliana. As it is evident from the result that stress and autophagy related proteins are highly co-expressed in the reference gene as well as in its orthologues, which reflects a strong association of these proteins in programmed cell death regulation of the plant. The programmed cell death observed in *acd11* is controlled by the hormone salicylic acid (SA) and can be inhibited by the expression of a bacterial SA hydroxylase (nahG) or by mutations in PAD4 and EDS1, as reported by Broderson *et al. (*2005) and Feys *et al*. (2001).

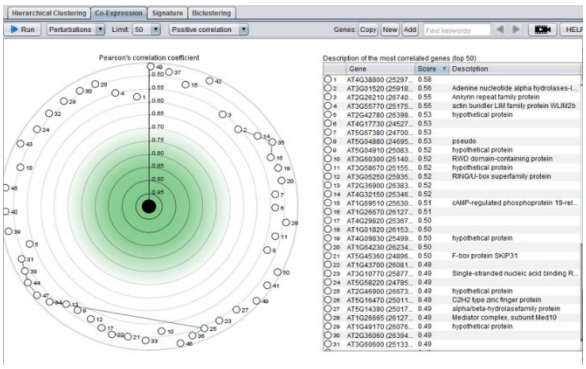


Figure 2*: Conditional stress co-expression analysis of reference gene ACD11 using Genevestigator co-expression tool.*

Table 2: *Co-expressed genes of the reference gene and its orthologues under stress conditions using Genevestigator tool.*

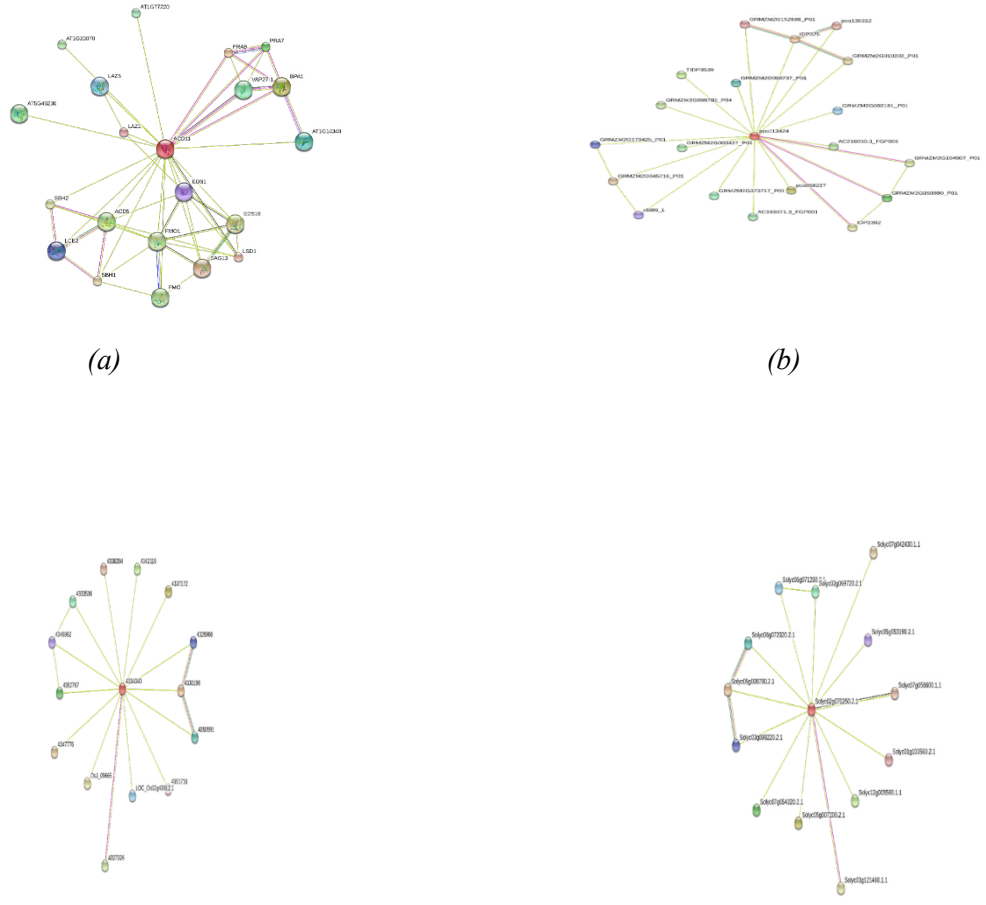
Protein	*Arabidopsis Thaliana*	*Glycine max*	*S.lycopersicum*	*Oryza sativa*	*Zea mays*
Syntaxin	AT4G17730 AT4G32150 AT1G266	GLYMA01G00760 GLYMA14G02120 GLYMA09G138	SOLYC05G013050 SOLYC01G100820 SOLYC03G1220	OS03G0137400	GRMZM5G806784

	70 AT3G60600 AT1G16240 AT2G36900	40 GLYMA16G08200 GLYMA02G42280	90 SOLYC02G032930		
UQ Ligase	AT4G09830 AT2G36060 AT5G32440	GLYMA10G43120 GLYMA06G02390	SOLYC04G080810	OS08G0170900 OS11G0483600 OS08G0540300	GRMZM2G171430
Zinc finger	AT5G16470	GLYMA14G16717 GLYMA12G13270 GLYMA10G43090 GLYMA07G12170	SOLYC03G063320 SOLYC09G008470	OS07G0167200 OS06G0697200	GRMZM2G049296 GRMZM2G000014
Stress related protein	AT1G3700	GLYMA10G28300 GLYMA03G41030	SOLYC09G090370 SOLYC01G080410 SOLYC09G0726	OS07G0589400 OS05G0122700	GRMZM2G106960

			70 SOLYC01G104600 SOLYC07G044850		
Autophagy related protein	AT2G45980	----------	SOLYC06G034160 SOLYC03G096790 SOLYC03G113000	OS03G0137400	GRMZM2G056572 GRMZM2G171435
Ras rekated protein	----------	GLYMA09G01950 GLYMA05G32520	SOLYC06G065230 SOLYC01G104700	----------	----------

Protein-protein interaction network(s) and 3D structure prediction

The PPI network of ACD11 and its orthologous genes was obtained from the STRING database to predict its interactions with other proteins. The analysis revealed ACD11's interaction with EDS1, LSD1, LAZ1, LCB, and FMO. LAZ1, which belongs to the DFU300 transmembrane protein family, regulates plant PCD associated with HR and is involved in acd11 cell death. Loss of LAZ1 function suppresses cell death in acd11 and certain types of HR cell death. The STRING database is a valuable tool for predicting protein interactions and understanding their functional impact (Malinovsky *et al*., 2010).



(a) *(b)*

(c) *(d)*

Figure 3: PPI of the reference gene and orthologues retrieved from STRING database network. (a) Arabidopsis Thaliana (b) Zea mayz (c) Lycopersicum esculentum (d) Oryza sativa.

node1	node2	node1 accession	node2 accession	node1 annotation	node2 annotation	score
ACD11	ACD5	AT2G34690.1	AT5G51290.1	*ACCELERATED CELL DEATH 11*	*ACCELERATED CELL DEATH 5*	0.902
ACD11	AT1G14340	AT2G34690.1	AT1G14340.1	*ACCELERATED CELL DEATH 11*	*RNA recognition motif-containing ...*	0.800
ACD11	AT1G23070	AT2G34690.1	AT1G23070.1	*ACCELERATED CELL DEATH 11*	*uncharacterized protein*	0.588
ACD11	AT1G77220	AT2G34690.1	AT1G77220.1	*ACCELERATED CELL DEATH 11*	*uncharacterized protein*	0.579
ACD11	AT5G45230	AT2G34690.1	AT5G45230.1	*ACCELERATED CELL DEATH 11*	*TIR-NBS-LRR class disease resist...*	0.580
ACD11	BPA1	AT2G34690.1	AT5G16840.2	*ACCELERATED CELL DEATH 11*	*binding partner of acd11 1*	0.938
ACD11	EDS1	AT2G34690.1	AT3G48090.1	*ACCELERATED CELL DEATH 11*	*enhanced disease susceptibility 1*	0.753
ACD11	EDS16	AT2G34690.1	AT1G74710.2	*ACCELERATED CELL DEATH 11*	*Isochorismate synthase 1; Involve...*	0.641
ACD11	FMO	AT2G34690.1	AT1G12200.1	*ACCELERATED CELL DEATH 11*	*flavin monooxygenase; Catalyzes ...*	0.597
ACD11	FMO1	AT2G34690.1	AT1G19250.1	*ACCELERATED CELL DEATH 11*	*flavin-dependent monooxygenase...*	0.597
ACD11	LAZ1	AT2G34690.1	AT4G38360.2	*ACCELERATED CELL DEATH 11*	*LAZARUS 1*	0.721
ACD11	LAZ5	AT2G34690.1	AT5G44870.1	*ACCELERATED CELL DEATH 11*	*LAZARUS 5*	0.759
ACD11	LCB2	AT2G34690.1	AT5G23670.1	*ACCELERATED CELL DEATH 11*	*long chain base2; Serine palmitoyl...*	0.758
ACD11	LSD1	AT2G34690.1	AT4G20380.8	*ACCELERATED CELL DEATH 11*	*zinc finger protein LSD1*	0.711
ACD11	PRA7	AT2G34690.1	AT1G55190.1	*ACCELERATED CELL DEATH 11*	*PRA1 family protein F2; May be in...*	0.894
ACD11	PRA8	AT2G34690.1	AT3G13720.1	*ACCELERATED CELL DEATH 11*	*PRA1 family protein F3; May be in...*	0.939
ACD11	SAG13	AT2G34690.1	AT2G29350.1	*ACCELERATED CELL DEATH 11*	*senescence-associated gene 13*	0.645
ACD11	SBH1	AT2G34690.1	AT1G69640.1	*ACCELERATED CELL DEATH 11*	*sphingoid base hydroxylase 1; Inv...*	0.645
ACD11	SBH2	AT2G34690.1	AT1G14290.1	*ACCELERATED CELL DEATH 11*	*sphingoid base hydroxylase 2; Inv...*	0.621
ACD11	VAP27-1	AT2G34690.1	AT3G60600.1	*ACCELERATED CELL DEATH 11*	*vesicle associated protein; May pl...*	0.861

Table 3. *Protein interacting partners of ACD11 and its orthologues*

	A.thaliana	*S.lycopersicum*	*G.max*	*P.trichocarpa*	*O.sativa*	*Zea mays*
EDS1	At3g48090	solyc06g071280	glyma04g34800	Poptr_0015s08100	Loc_os09g22450	grmzm2g045716
LSD1	at4g20380	solyc02g069720	glyma05g30490	Poptr_0011s15810	Loc_os12g41700	grmzm2g114613
LAZ1	at4g38360	solyc07g054320	glyma06g00300	10Poptr_0009s167	Loc_os06g51100	grmzm2g000427
LCB2	at5g23670	solyc06g072320	glyma02g07250	Poptr_0012s10630	Loc_os01g70380	grmzm2g010202
FMO	at1g12200	solyc07g042430	glyma17g05160	Poptr_0001s36280	Loc_os03g08410	grmzm2g000427

Phyre2 was used to predict the 3D structure of the reference protein and its orthologues, and HHPred was used to validate the structure by comparing the secondary structure of the reference protein to that of its orthologues. Although ACD11 has low sequence homology with other GLTP homologs, crystallographic data has shown conserved structural homology between Arabidopsis ACD11, human GLTP, and human CPTP. The 3D model for ACD11 was retrieved from the PDB database with ID 4NT1.

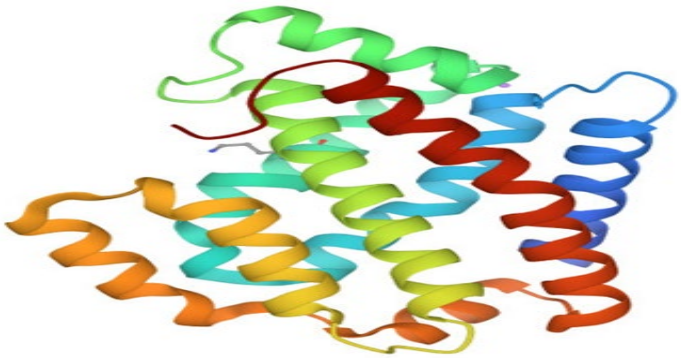


Figure 4: *3D Structure of the ACD11 retrieved from Phyre2*

Prediction of ACD11 protein disorder and ligand binding residue and its characterization

It is noted that DisEMBL is a web-based tool used to study and predict Intrinsic Disorder Proteins (IDPs), and that understanding protein function and folding pathways is important for protein disorder. IDPs are thought to become ordered only when bound to another molecule or when biochemical environment changes. Loops/coils are not necessarily disordered, but protein disorder is only found within loops. Disordered regions in a protein's flexibility facilitate different conformational possibilities and are therefore important for functionality as these regions may contain functional sites or linear motifs. The functional diversity of the first and middle segments of the protein structure is revealed by Disorder by Hot-loops and Loop-coils.

Table 4*. Protein disorder by Hot-loop*

S.NO	PROTEIN	RESIDUE POSITION	RESIDUES
1	*A.thaliana*	1-9, 92-107, 121-131, 199-206	MADSEADKP, RKAGSHTRNLLRVKRG, SEGDNSLKDPA, SKQLGIDW
2	*B.rapa*	**1-19**, 32-47, 62-73	**MDYVAKVEDLAKASSSVST**, CVRKAGSHTRNLLRVK, ASEGDNSLKDPA

3	*S.lycopersicum*	1-11, 99-112, 126-136, 201-210	MANHVAEEKPL, AGSHTRNLLRVKRG, SEGNSLKDPAS, FTSRDLGTDW
4	*G.max*	1-8, 92-111, 124-132	MTEGNGDK, NTVRKGGSHTRNLLRVKRGL, TEGNSLRDP
5	*V.vinifera*	86-104, 120-127	IEGNCVRKAGSNSRNLLRV, ASDDNSLR
6	*O.sativa*	1-10, 77-108, 183-192	MGSSDGDKPL, ISKLPEMVELDIQKGTVRQAGSHT RNMLRVKR, FIRASGPVIL
7	*Z.mays*	1-14, 22-59, 126-161, 233-258	MNALKHRKARQHQ, QKNKKGEGKNSRLLPRACQ HPRSASSPKAAMGSSQAD, KSISTLPSMVERDIQ TDTVRKPGSHTRNLLRVKRGI, NFVRSSAP VICYVDDLFTSRNLGIDW
8	*P.trichocarpa*	1-10, 73-107	MGDLETEKPL, KSIGTLQSVLDKDVERNS VRKGGSHSRNLLRVKRG

Table 5. *Protein disorder by Loop/coil*

S.NO	PROTEIN	RESIDUE POSITION	RESIDUES
1	*A.thaliana*	1-8, 24-36, 121-141, 194-206	MADSEADK, VNSPNPEVPVTQF, SEGDNSLKDPATKSYAQVFA, DNLFLSKQLGIDW

2	*B.rapa*	63-85	SEGDNSLK DPASKSYDQV FRPHH
3	*S.lycopersicum*	1-9, 30-49, 126-145, 201-210	MANHVAEEK, LDEAAKMEVAPFSHACTLVS, SEGNS LKDPASKAYTQVFAP, FTSRDLGTDW
4	*G.max*	1-8, 22-53, 92-104, 125-136	MTEGNGDK, NVFTDSQSAEAEVKVAPFSHACSLVSPLFGC L, NTVRKGGSHTRNL, EGNSLRDPASKA
5	*V.vinifera*	26-50, 83-103, 120-133	SQTVDIEVAPFSHACSLVSPLFGCL, DHDIEGNC VRKAGSNSRNLLR, ASDDNSLRNPASTA
6	*O.sativa*	1-9, 26-36, 77-103, 199-207	MGSSDGDKP, QQAPGPAMEVG, ISKLPEMVELDIQK GTVRQAGSHTRNM, TSRNLGMDW
7	*Z.mays*	1-10, 24-61, 77-89, 127-155, 205-212, 235-258	MNALKHRKAS, NKKGEGKNSRLLPRACQ HPRSASSPKAAAMGSSQADKP, KQQPAVPMDAGAF, SISTLPSMVERDIQTDTVRKPGSHTRNLL, VRSSAP VICYVDDLFTSRNLGIDW
8	*P.trichocarpa*	1-8, 30-50, 83-102, 121-139, 197-209	MGDLETEKPL, KSIGTLQSVLDKDVERNS VRKGGSHSRNLLRVKRG

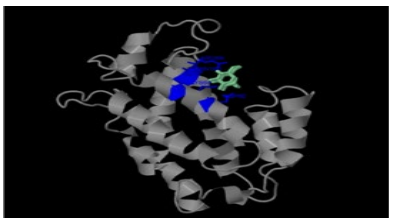
Table 6. *Protein disorder by the method of beta-aggregation*

S.NO	PROTEIN	RESIDUE POSITION	RESIDUES
1	*A.thaliana*	20-24, 75-82, 108-115	LAIIV, ISTLVVMM, LDMVKVLF

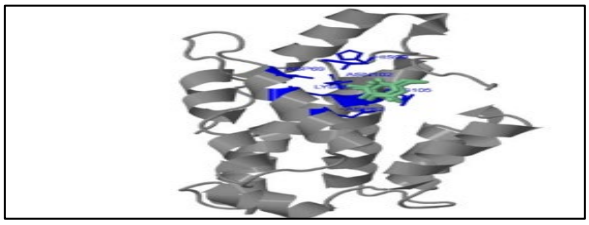
2	*B.rapa*	17-24, 131-135	VSTLVVMM, VIAYL,
3	*S.lycopersicum*	113-120, 192-202	LDMVKVLF, TVILYIDKLFT
4	*G.max*	113-118, 191-200	MVRVLF, LIQYIDKLFV
5	*V.vinifera*	188-192	VILYI
6	*O.sativa*	45-56, 110-121, 190-194	VSVLFGCLGIAF, IDMVKILFEQIL, VILYV
7	*Z.mays*	65-69, 96-107, 161-172	IAVSF, VSVLFGCLGIAF, IDMVKVLFEQIL
8	*P.trichocarpa*	206-217	QSTFYLTWCTII

Ligand Binding residues of the reference protein ACD11 and its orthologues were predicted through RaptorX. The residues involved in forming the binding pockets for the ligands are given in Table 3.

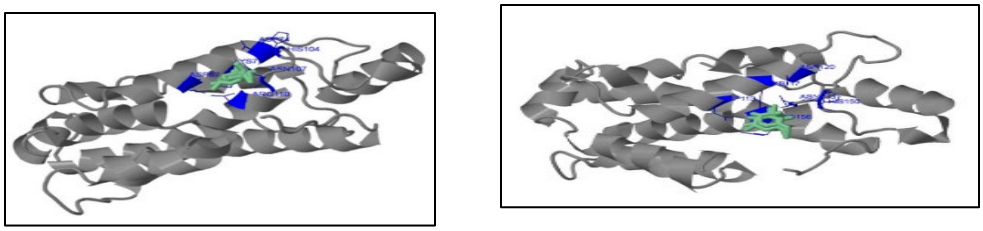
EIS(N-{(2S,3R,4E)-3-hydroxy-1-[(3-O-sulfo-beta-D-galactopyranosyl) oxy]octadec-4-en-2-yl}dodecanamide) binding residues of acd11 and its orthologous protein model obtained from RaptorX server are shown in figure 4. The residues in the binding site pockets are the same for ACD11 and its orthologues. In active site pocket of the reference protein and its orthologues the most prominent and interactive amino acids are Asparagine and Histidine ranging at the same position as shown in the table. 7



(a)



(b)



(c) (d)

Figure 5: *Ligand binding site and residue prediction of the reference protein and its orthologues retrieved from RaptorX. (a) Arabidopsis thaliana (b) Glycine max (c) Oryza sativa (d) Zea mayz*

Table 7. *Binding residues and their score*

	A.thaliana	*G.max*	*O.sativa*	*Z.mays*
	60ASP 0.66	74ASP 0.68	69ASP 0.70	120ASP 0.66
Binding residues	97HIS 0.67	104HIS 0.66	99HIS 0.66	150HIS 0.64

And score

CONCLUSION

In the present study we identified, predicted and comprehensively analyzed the accelerated cell death protein ACD11 of Arabidopsis thaliana and its orthologues like *Glycine max, Poplus trichocarpa, Solanum lycopersicum* form dicot plants and *Oryza sativa* and *Zea mays* from monocots across the diverse group of plant species. By taking advantage of the available computational tools, we perform complete analysis of developmental expression, protein-protein interaction, Co-expression, protein structure prediction and determination of intrinsic protein disorder, and finally ligand residues of the selected proteins. It is obvious form the result analysis that ACD11 and its orthologues mostly express in the last developmental stages of the plant i.e. senescence, which is confirming the role of ACD11 in cell death mechanism of the plant and other associated pathways.

From the study of the Co-expression the result shows that some the membrane bounding proteins SNARE, disease resistance and Autophagy related genes are co-expressed with acd11 and their orthologues, which validate again that during the cell death response either due to biotic or abiotic stress the disease resistance and autophagy related genes are also regulated. Form protein-protein interaction it is clear that the

interacting partners of the ACD11 and their orthologues from diverse plants species is Enhanced disease resistance protein EDS1and LAZ1, so it is evident from both the results that there is a strong relationship between the co-expressed and the interacting proteins leading to a regulatory pathway of ACD11. Intrinsic disorder protein study predicts the flexibility in protein conformation from which it can be assumed that this protein has the capability of diverse functioning properties. From ligand binding study it is deduced that the common residues in the active site pocket of the protein are ASPRGINE and HISTDINE with high score of average distance and contact of the residue. Future work will elaborate more about the interacting protein partners and co-expressed proteins to determine a common gene-regulatory network of ACD11 and its role in programmed cell death.

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