

Detailed *In Silico* Analysis of *Arabidopsis* N-myc Downregulated Like (NDL) Interactome with Reference to Stress Sensing

Abhishek Kanojia and Yashwanti Mudgil*

Department of Botany, North Campus, University of Delhi, Delhi-110007

ABSTRACT

Proteins are main players of almost all biological activities, performing their function by interacting with other proteins. These protein-protein interactions act as fundamental basis for signalling in living organisms. These interactions result in formation of complex protein networks with important roles in plant growth and development and in various stress responses. For instance, heterotrimeric G proteins (G α monomer and G $\beta\gamma$ dimer) are key proteins associated with the various growth and development related processes like germination, light response, shoot and root growth, stomatal regulation and stress responses. N-myc Downregulated Like proteins or NDLs are downstream components of the G protein signalling pathway. Expression of their animal homologs, NDRG proteins, is known to be induced in various stress conditions like hypoxia, metal ions and reducing agents. NDL proteins were isolated in a yeast two hybrid screen designed to isolate interacting partners of the G $\beta\gamma$ dimer. Later, NDL was found to interact with more than sixty other proteins in a comprehensive screen to elucidate the signalling pathway related to G proteins. The study of a complete set of interactors of a protein under specific condition (interactome) can help us to understand the molecular mechanism of action or its functional mapping. In the present study, putative interactors (PIs) of NDL proteins were analysed *in silico*, in terms of their functions and expression in different plant parts and stress conditions (mostly abiotic). We found that more than fifty percent of the PIs of the NDL interactome showed both, tissue specific expressions and responses to various stress conditions. This study can be used to plan experiments to elucidate the molecular mechanism of plant stress response.

Key words: abiotic, *Arabidopsis*, biotic, downstream effectors, gene expression analysis, interactome, interactor.

***Author for Correspondence:** Yashwanti Mudgil; Email: ymudgil@gmail.com, ymudgil@botany.du.ac.in

Introduction

Different plants have varying potential to tolerate various stresses; this tolerance is mainly based on their genetic constitution. Stress exposure in plants results in changes in their physiological and metabolic processes which in turn affect their growth and development. Agriculture is adversely affected by many problems linked to abiotic and biotic stresses, e.g., shortage of water, high/low temperatures and salinity, resulting in huge agro-economic losses.

G proteins are identified as key proteins associated with the various growth and development related processes like germination, light response, shoot and root growth, and stomatal regulation (Lease et al., 2001; Ullah et al., 2003; Pandey et al., 2006; Trusov et al., 2007; Zhang et al., 2008; Liu et al., 2012; Ming et al., 2015). G protein components are also involved in response to stress conditions like drought, heat, cold, ozone and salt (Joo et al., 2005; Ming et al., 2015; Chakraborty et al., 2015; Xu et al. 2015). In *Arabidopsis thaliana* (*Arabidopsis*), G proteins are known to occur as hetero-trimeric subunits, G α , G β

and G γ (Ma et al., 1990; Weiss et al., 1994; Mason & Botella 2001; Choudhury et al., 2019). The molecular mechanism of action of very few candidate genes working downstream in the G protein signalling is known. NDL1 is one such effector discovered in a screen for the interactors of $\beta\gamma$ dimer in *Arabidopsis*. Studies of protein interacting with G protein core components and selected effectors (interactome study using yeast two hybrid and bimolecular fluorescence complementation) divulged important evidence about interactions of the G protein. About sixty plus putative interactors (PIs) of NDL proteins are known today (Mudgil et al., 2009; Klopffleisch et al., 2011; Khatri et al., 2015).

NDL are alpha/beta hydrolase fold comprising proteins, functional characterization studies using overexpression/ downregulation of these genes showed that they function as positive regulators of primary root and shoot growth and maintain proper architecture of the plant by modulating auxin transport (Mudgil et al., 2009; Mudgil et al., 2013). Approximately thirty *NDL1/NDLI* genes/proteins have been reported in various

plant species till date (NCBI Research Coordinators, 2016). Expression of NDRGs, the animal homolog of NDL protein, is reported to be induced by many stress conditions like hypoxia, DNA damage, metal ions etc. indicating their probable involvement during various stress responses (Piquemal et al., 1999; Kovacevic and Richardson 2006; Melotte et al., 2010).

Recently it has been found that during water deficient conditions expression of *NDL1* positively correlated with the increase in the rate of transpiration and projected rosette area in *Arabidopsis* (Rymaszewski et al., 2017). Our recent comparative expression profiling of three members of the *NDL* family in both presence and absence of *AGB1* showed different responses - *NDL1* expression showed changes in patterns, whereas expression patterns of *NDL2* and *NDL3* were not affected much during salinity stress. The responses of *NDL-AGB1* modules response towards abiotic stress and hormone treatments suggest that each member plays a different role under different kind of abiotic stresses, and also establish their possible role and use as stress markers (Katiyar and Mudgil, 2019). Further investigations are required to reveal detailed molecular characterization of *NDL* interactors under abiotic stress to reveal their function in plants. The current study of *NDLs* is a step forward in that direction.

Materials and Methods

G Protein and NDL1 Interactome

The biological general repository for interaction datasets BIOGRID (<https://thebiogrid.org/21021/summary/Arabidopsis-thaliana/ndl1.html>) was used to get interaction information about all the PIs (=interactome) of all the subunits of G protein complex and *NDL1* in *Arabidopsis*.

Network and Expression Analysis of Putative Interactors (PIs)

Information on the expression and response, under various abiotic stresses, of all the PIs of *NDL1* from the G protein interactome was collected using the gene ontology feature in NCBI (<https://www.ncbi.gov>). Based on the information gathered, networks were created using yEd graph generator (<http://www.yworks.com/products/yed>).

Expression analysis of various PIs was performed using eFP browser also known as Electronic Fluorescent Pictograph by TAIR, <http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi> (Winter et al., 2007). Expression data for PIs

in different abiotic-stress conditions (heat, cold, drought, salt, osmotic, genotoxic, oxidative, UV-B, wounding); hormones (auxin, abscisic acid, cytokinin, ethylene, gibberellic acid, brassinolides, brassinosteroids); biotic stresses (infected with *Botrytis cineria*, *Pseudomonas syringae*, *Phytophthora infestans*, *Erysiphe orontii*, elicitors) were obtained as fold change values at different time intervals for comprehensive analysis.

Expression Analysis in Different Parts of Plant

Electronic Fluorescent Pictograph by TAIR (<http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi>), was used to retrieve the expression data of PIs in different plant parts.

Results

Interactome Analysis of NDL and of G Protein Subunits

G protein interactome analysis showed that all the subunits of G protein interact with other interactors (proteins), which were found to be involved in diverse functions in plants (Table 1). This supports the involvement of G protein in different functions. G protein subunits share some PIs (Figure 1A) namely, *AGB1*, *PLDA1* (Phospholipase D alpha 1), *FSD1* (Fe superoxide dismutase 1), *VOZ1* (Vascular plant one zinc finger protein 1), *VOZ2* (Vascular plant one zinc finger protein 2) interact with *GPA1* and *AGB1*; *AGB1* interacts with *GPA1* and *NDL1*; *ATARCA* (Transducin/WD40 repeat-like superfamily protein) interacts with *AGB1*, *AGG1* and *AGG2*; *BAK1* (Leu-rich receptor Serine/threonine protein kinase), *BIR1* (BAK1-interacting receptor-like kinase), *CERK1* (Chitin elicitor receptor kinase 1) interact with *GPA1*, *AGG1* and *AGG2*; *FTA* (Farnesyltransferase A), *NUDT7* (Nudix hydrolase 7), *PGGT1* (Geranylgeranyl transferase type-1 subunit beta) interact with *AGG1* and *AGG2*; *XLG2* (Extra-large GTP-binding protein 2) interacts with *AGB1*, *AGG1*, *AGG2* and *AGG3*; and *SYP23* (Syntaxin-31) interacts with *GPA1* and *NDL1*.

PIs Related to Abiotic Stress and Network Analysis of NDL1 Interactome

The literature and online data base (NCBI) were thoroughly searched to find information related to interactor responses to abiotic stresses. It was found that 24 interactors out of 56 of *GPA1*, 23 out of 95 of *AGB1*, 7 out of 12 of *AGG1*, 6 out of 9 of *AGG2*, 1 of 1 of *AGG3* and 31 out of 62 of *NDL1* showed response (in terms of mutant analysis/ expression analysis/ direct assays/ genetic interaction) to various

1A.

GENE ID	INTERACTORS	GPA1	AGB1	AGG1	AGG2	AGG3	NDL1
AT3G63420	AGG1	✓	✓	x	X	X	✓
AT4G34460	AGB1	✓	x	✓	x	x	✓
AT1G18080	ATARCA	x	✓	✓	✓	x	x
AT4G33430	BAKI	✓	x	✓	✓	x	x
AT5G48380	BIR1	✓	x	✓	✓	x	x
AT3G21630	CERK1	✓	x	✓	✓	x	x
AT3G59380	FTA	x	x	✓	✓	x	x
AT4G12720	NUDT7	x	x	✓	✓	x	x
AT3G15730	PLDA1	✓	✓	x	x	x	x
AT2G39550	PGGT1	x	x	✓	✓	x	x
AT4G34390	XLG2	x	✓	✓	✓	✓	x
AT4G25100	FSD1	✓	✓	x	x	x	x
AT1G38520	VOZ1	✓	✓	x	x	x	x
AT2G42400	VOZ2	✓	✓	x	x	x	x
AT4G17730	SYP23	✓	x	x	x	x	✓

1B.

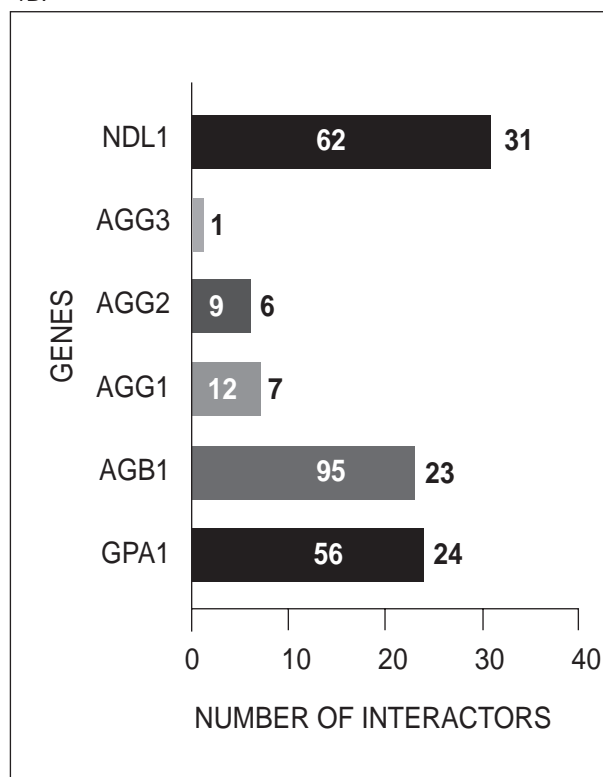


Figure 1. (A) List of interactors shared between heterotrimeric G protein subunits and NDL1; (B) Numbers of putative interactors of G protein heterotrimeric subunits (GPA1, AGB1, AGG1, AGG2, AGG3) and NDL1 interactomes showing response to various abiotic stresses, as a fraction of the total number of interactors (written in white within the bars) in each interactome.

abiotic stress conditions (Figure 1B and Table 1). This further indicates that many of the PIs of G protein as well as of NDL1 are potentially involved in stress-management in plants.

The information gathered was used for creating networks for NDL1 protein PIs (Figure 2). Network analysis showed that NDL1 protein PIs are involved in various plant processes and functions (Figure 2A and 2B). Process-network revealed that 5 PIs are involved in responses to biotic stress, 6 in signalling, 3 in cell cycle, 7 in gene expression, 6 in hormonal response, 17 in developmental role, 24 in metabolic or biosynthesis processes, 31 in abiotic stress response, 11 in other functions not included in above categories and 9 PIs have unknown role (Figure 2A).

Function-network revealed that, of the 62 PIs of NDL1, the majority (29) are involved in enzymatic functions, 18 in protein binding, 8 in metal binding, 7 in nucleic acid binding, 3 in oxidation-reduction, 2 have receptor function, 4 in other functions while 16 PIs have unknown functions (Figure 2B).

Expression of PIs of NDL1 in Different Parts of the Plant

Gene expression varies in different parts of plants. We retrieved the absolute expression data (Table 2) of PIs in different plant parts and found that of all the PIs, *MT2A* showed maximum expression in dry seed, cauline leaf, flower bud, flower, senescence leaf, siliques; *CA1* in cotyledon, rosette; *ANNAT1* in hypocotyl; *RS27A* in root; *ATMS1* in stem; *P14GAMMA4* in pollen (however *NDL1* showed highest expression in pollens); *LOX2* in vegetative rosette. If we see expression of particular gene among all the parts then, *ADS2* showed highest expression in dry seed; *AGB1* in senescing leaf; *ALDH11A3* in stem; *ANNAT1* in hypocotyl; *ABHF* in pollen; *CD1BP* in dry seed; *TIR900* and *TIR920* in cotyledon; *UDPGTSF* in stem; *DUF2358* in cauline leaf; *DNARALP* in dry seed; *RPSA3* in vegetative rosette; *PE/GH* in vegetative rosette; *HP* in flower; *NUO* in root; *CYS4* in siliques; *NUCLEASE* in root; *PEARLI4* in senescing leaf; *PLCL* in cotyledon; *HSFP* in siliques; *TWD40_2* in stem; *DUF1162* in senescing

Table 1. Putative Interactors (PIs) of G protein subunits and NDL1, interactors highlighted in grey colour are published and have response towards abiotic stress.

G protein subunits and NDL1 interactors description					
Interactors of GPA1					
S.No.	Name	Description	S.No.	Name	Description
1	PSB29	protein THYLAKOID FORMATION 1	29	AT5G13440	ubiquinol-cytochrome C reductase iron-sulfur subunit
2	GCR1	G-protein-coupled receptor 1	30	AT5G23280	transcription factor TCP7
3	PRN	pirin-1	31	AT5G24620	pathogenesis-related thaumatin-like protein
4	RGS1	putative membrane receptor protein RGS1	32	AT5G26790	hypothetical protein
5	BAK1	Leu-rich receptor Serine/threonine protein kinase	33	AT5G61820	hypothetical protein
6	BIR1	BAK1-interacting receptor-like kinase BIR1	34	BAG6	BCL-2-associated athanogene 6
7	CERK1	chitin elicitor receptor kinase 1	35	CAS	calcium sensing receptor
8	GPCR	G-protein coupled receptor 2	36	CAT9	cationic amino acid transporter 9
9	GTG1	GPCR-type G protein 1	37	CPK1	calcium dependent protein kinase 1
10	GTG2	GPCR-type G protein 2	38	CSN5A	COP9 signalosome complex subunit 5a
11	PD1	arogenate dehydratase 3	39	EIF3A	eukaryotic translation initiation factor 3A
12	PLDALPHA1	phospholipase D alpha 1	40	FSD1	superoxide dismutase [Fe]
13	ADT1	arogenate dehydratase 1	41	GDU2	glutamine dumper 2
14	AGB1	guanine nucleotide-binding protein subunit beta	42	KAC1	kinesin CDKA;1 associated 1
15	AGP16	arabinogalactan protein 16	43	LOS1	elongation factor EF-2-like protein LOS1
16	ARD4	acireductone dioxygenase 4	44	LRR1	leucine-rich repeat protein 1
17	AT1G07210	ribosomal protein S18	45	MYB32	transcription factor MYB32
18	AT1G09995	DNA repair DEAD helicase RAD3/XP-D subfamily protein	46	NDPK2	nucleoside diphosphate kinase II
19	AT2G21580	40S ribosomal protein S25-2	47	PDF1B	peptide deformylase 1B
20	AT2G45680	transcription factor TCP9	48	RD2	desiccation responsive protein
21	AT3G24120	myb family transcription factor	49	SPX1	SPX domain-containing protein 1
22	AT3G52570	alpha/beta-Hydrolases superfamily protein	50	SYP23	syntaxin-23
23	AT3G61480	beta-chain like quinoprotein amine dehydrogenase	51	TCP16	transcription factor TCP16
24	AT4G00752	UBX domain-containing protein	52	TCP3	TEOSINTE BRANCHED 1, cycloidea and PCF transcription factor 3
25	AT4G26410	hypothetical protein	53	UNE16	unfertilized embryo sac 16 protein / transcription factor
26	AT4G33060	cyclophilin-like peptidyl-prolyl cis-trans isomerase family protein	54	VOZ1	vascular plant one zinc finger protein
27	AT4G37180	myb family transcription factor	55	VOZ2	vascular plant one zinc finger protein 2
28	AT5G13430	ubiquinol-cytochrome C reductase iron-sulfur subunit	56	XTH4	endoxyloglucan transferase A1
Interactors of AGB1					
1	AGG1	guanine nucleotide-binding protein subunit gamma 1	11	CMPG1	ubiquitin-protein ligase CMPG1
2	ARD1	acireductone dioxygenase 1	12	NDL1	N-MYC downregulated-like 1 protein
3	MPK6	MAP kinase 6	13	RACK1B_AT	receptor for activated C kinase 1B
4	NPH3	root phototropism protein 3	14	RACK1C_AT	receptor for activated C kinase 1C
5	AGG2	G-protein gamma subunit 2	15	ACR7	ACT domain repeat 7 protein
6	AT4G03415	putative protein phosphatase 2C 52	16	AGP24	arabinogalactan protein 24
7	VIP1	VIRE2-interacting protein 1	17	ALDH3F1	aldehyde dehydrogenase 3F1
8	ZIP4	protein ZIG SUPPRESSOR 4	18	ASHR2	histone-lysine N-methyltransferase ASHR2
9	ATARCA	guanine nucleotide-binding protein subunit beta-like protein A	19	AT1G02870	hypothetical protein
10	BIN2	Shaggy-related protein kinase eta	20	AT1G05000	atypical dual-specificity phosphatase 1

S.No.	Name	Description	S.No.	Name	Description
21	AT1G20970	hypothetical protein	58	ATNTT2	ADP, ATP carrier protein 2
22	AT1G27530	ubiquitin-fold modifier-conjugating enzyme 1	59	BPA1	binding partner of acd11 1
23	AT1G33055	hypothetical protein	60	CDC48B	cell division control protein 48-B
24	AT1G49870	hypothetical protein	61	CER2	fatty acid elongation machinery component ECERIFERUM2
25	AT1G54290	protein translation factor SUI1-2	62	DDB1A	DNA damage-binding protein 1a
26	AT1G65010	flower development-related protein	63	EMB975	pentatricopeptide repeat-containing protein
27	AT2G19270	hypothetical protein	64	FSD1	superoxide dismutase [Fe]
28	AT2G19460	hypothetical protein	65	GDU2	glutamine dumper 2
29	AT2G23070	casein kinase 2, alpha polypeptide	66	GP ALPHA 1	guanine nucleotide-binding protein alpha-1 subunit
30	AT2G24420	ATPase-related DNA repair protein	67	GRF2	14-3-3-like protein GF14 omega
31	AT2G46535	hypothetical protein	68	GRF3	14-3-3-like protein GF14 psi
32	AT3G03890	FMN binding protein	69	GRP2	glycine rich protein 2
33	AT3G05920	heavy-metal-associated domain-containing protein	70	IQD6	IQ-domain 6 protein
34	AT3G11850	hypothetical protein	71	LOS2	bifunctional enolase 2/transcriptional activator
35	AT3G12180	protein cornichon-like1	72	LYSOPL2	lysophospholipase 2
36	AT3G14160	2-oxoglutarate-dependent dioxygenase family protein	73	MC8	metacaspase 8
37	AT3G19370	hypothetical protein	74	NAC014	protein NAC 014
38	AT3G23450	hypothetical protein	75	NAC069	NAC transcription factor
39	AT3G24120	myb family transcription factor	76	NAC089	membrane-tethered transcription factor ANAC089
40	AT4G07820	cysteine-rich secretory proteins, antigen 5 and pathogenesis-related protein 1 domain-containing protein	77	NQR	oxidative stress tolerance protein NQR
41	AT4G16240	hypothetical protein	78	NTL9	NAC transcription factor-like 9
42	AT4G25390	receptor-like serine/threonine-protein kinase	79	PAP3	purple acid phosphatase 3
43	AT4G29020	glycine-rich protein	80	PHR2	photolyase/blue-light receptor 2
44	AT4G32175	PNAS-3 related protein	81	PLDALPHA1	phospholipase D alpha 1
45	AT5G01010	hypothetical protein	82	PVA12	VAP-like protein 12
46	AT5G08510	pentatricopeptide repeat-containing protein	83	RR1	response regulator 1
47	AT5G10920	putative argininosuccinate lyase	84	SBP2	selenium-binding protein 2
48	AT5G18400	anamorsin homolog	85	SHM3	serine hydroxymethyltransferase 3
49	AT5G19960	RNA recognition motif-containing protein	86	SYP31	syntaxin-31
50	AT5G24620	pathogenesis-related thaumatin-like protein	87	TFIIIA	transcription factor IIIA
51	AT5G27330	Prefoldin chaperone subunit family protein	88	TOM2A	tobamovirus multiplication 2A protein
52	AT5G51770	protein kinase family protein	89	VAP	vesicle associated protein
53	AT5G59550	ABA- and drought-induced RING-DUF1117 protein	90	VAP27-2	vamp/synaptobrevin-associated protein 27-2
54	AT5G59650	probable LRR receptor-like serine/threonine-protein kinase PAM74	91	VOZ1	vascular plant one zinc finger protein
55	AT5G66180	S-adenosyl-L-methionine-dependent methyltransferase domain-containing protein	92	VOZ2	vascular plant one zinc finger protein 2
56	ATBFRUCT1	beta-fructofuranosidase, insoluble isoenzyme CWINV1	93	VPS60.1	vacuolar protein sorting protein 60.1
57	ATKDSA1	2-dehydro-3-deoxyphosphooctonate aldolase 1	94	XBCP3	papain-like cysteine peptidase
			95	XLG2	extra-large GTP-binding protein 2

S.No.	Name	Description	S.No.	Name	Description
Interactors of AGG1					
1	AGB1	guanine nucleotide-binding protein subunit beta	7	FTA	farnesyltransferase A
2	ATARCA	guanine nucleotide-binding protein subunit beta-like protein A	8	GONST1	GDP-mannose transporter GONST1
3	BAK1	Leu-rich receptor Serine/threonine protein kinase BAK1	9	MLO4	MLO-like protein 4
4	BIR1	BAK1-interacting receptor-like kinase BIR1	10	NUDT7	nudix hydrolase 7
5	CERK1	chitin elicitor receptor kinase 1	11	PGGT-I	geranylgeranyl transferase type-1 subunit beta
6	CNGC17	cyclic nucleotide-gated channel 17	12	XLG2	extra-large GTP-binding protein 2
Interactors of AGG2					
1	AGB1	guanine nucleotide-binding protein subunit beta	6	FTA	farnesyltransferase A
2	ATARCA	guanine nucleotide-binding protein subunit beta-like protein A	7	NUDT7	nudix hydrolase 7
3	BAK1	Leu-rich receptor Serine/threonine protein kinase BAK1	8	PGGT-I	geranylgeranyl transferase type-1 subunit beta
4	BIR1	BAK1-interacting receptor-like kinase BIR1	9	XLG2	extra-large GTP-binding protein 2
5	CERK1	chitin elicitor receptor kinase 1			
Interactors of AGG3					
1	XLG2	extra-large GTP-binding protein 2			
Interactors of NDL1					
1	ADS2	16:0 delta9 desaturase 2	23	AT5G25100	Endomembrane protein 70 protein family
2	AGB1	GTP binding protein beta 1	24	VQ32	VQ motif-containing protein
3	ALDH11A3	Aldehyde dehydrogenase 11A3	25	AT5G63370	Protein kinase superfamily protein
4	ANNAT1	Annexin 1	26	ATMS1	Cobalamin-independent synthase family protein
5	AT1G10740	Alpha/beta-Hydrolases superfamily protein	27	BOB1	HSP20-like chaperones superfamily protein
6	AT1G22970	Cyclin-D1-binding protein	28	BPA1	Binding partner of acd11 1
7	AT1G72900	Toll-Interleukin-Resistance (TIR) domain-containing protein	29	CA1	carbonic anhydrase 1
8	AT1G72920	Toll-Interleukin-Resistance (TIR) domain family protein	30	CAD9	cinnamyl alcohol dehydrogenase 9
9	AT1G75420	UDP-Glycosyltransferase superfamily protein	31	CHL11	P-loop containing nucleoside triphosphate hydrolases superfamily protein
10	AT1G79510	Hypothetical protein (DUF2358)	32	CKS2	CDK-subunit 2
11	AT2G24420	DNA repair ATPase-like protein	33	COB	COBRA-like extracellular glycosyl-phosphatidyl inositol-anchored protein family
12	AT3G04840	Ribosomal protein S3Ae	34	EIF4A-III	eukaryotic initiation factor 4A-III
13	AT3G62460	Putative endonuclease or glycosyl hydrolase	35	HMGB3	high mobility group B3
14	AT4G16240	Hypothetical protein	36	HOT5	GroES-like zinc-binding dehydrogenase family protein
15	AT4G16450	NADH-ubiquinone oxidoreductase	37	IDH-V	isocitrate dehydrogenase V
16	AT4G16500	Cystatin/monellin superfamily protein	38	KCS9 3	3-ketoacyl-CoA synthase 9
17	AT4G29780	Nuclease	39	KLCR1	Tetratricopeptide repeat (TPR)-like superfamily protein
18	AT4G38550	Phospholipase-like protein (PEARLI 4) family protein	40	LOX2	Lipoxygenase 2
19	AT4G38690	PLC-like phosphodiesterases superfamily protein	41	LSD1	LSD1 zinc finger family protein
20	AT4G40030	Histone superfamily protein	42	MT2A	Metallothionein 2A
21	AT5G24710	Transducin/WD40 repeat-like superfamily protein	43	OASA1	O-acetylserine (thiol) lyase (OAS-TL)
22	AT5G24740	Vacuolar protein sorting-associated protein, putative (DUF1162)	44	PAB1	Proteasome subunit PAB1

S.No.	Name	Description	S.No.	Name	Description
45	PDR2	Phosphate deficiency response 2	54	SYP23	Syntaxin of plants 23
46	PI4K GAMMA 4	Phosphoinositide 4-kinase gamma 4	55	THM1	Thioredoxin M-type 1
47	PP2AA2	Protein phosphatase 2A subunit A2	56	TIM10	Tim10/DDP family zinc finger protein
48	PPa4	Pyrophosphorylase 4	57	TUA2	Tubulin alpha-2 chain
49	ROC3	Rotamase CYP 3	58	UBP6	Ubiquitin-specific protease 6
50	RS27A	Ribosomal protein S27	59	XBAT32	Hypothetical protein
51	SLP3	Subtilisin-like serine protease 3	60	XT1	Xylosyltransferase 1
52	SLT1	HSP20-like chaperones superfamily protein	61	YAB2	Plant-specific transcription factor YABBY family protein
53	SPPL2	Signal peptidase like 2	62	RAD5	DNA/RNA helicase protein

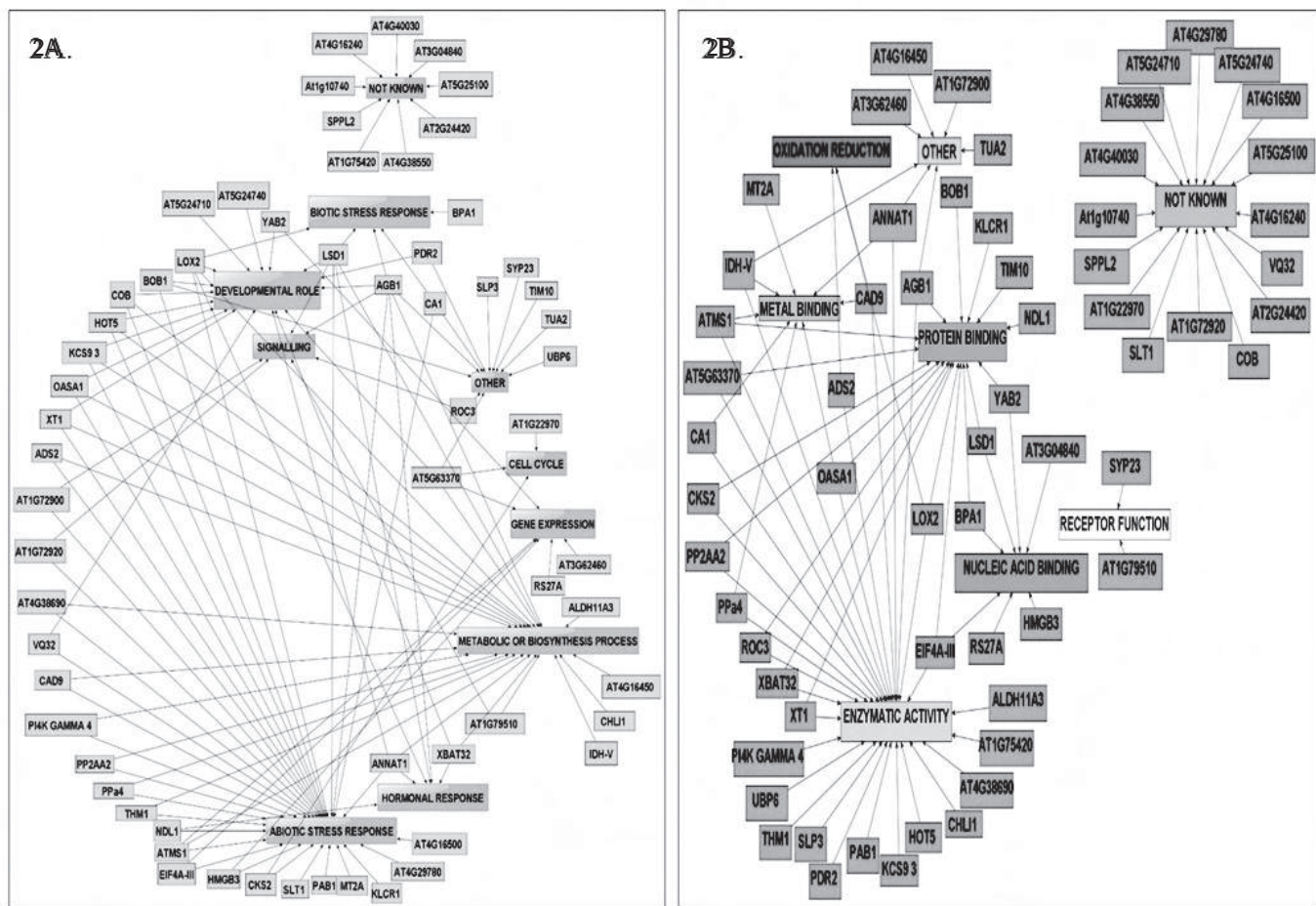


Figure 2: Processes and functions network analysis- the gene ontology feature of NCBI was used for collecting information to create these graphs. Networks showing putative interactors of NDL1 involved in various plant (A) processes and (B) functions.

leaf; *EP70* in stem; *VQ32* in root; *PKSFP* in pollen; *ATMS1* in stem; *BOB1* in dry seed; *BPA1* in dry seed; *CA1* in cotyledon; *CAD9* in flower bud; *CHL1* in vegetative rosette; *CKS2* in pollen; *COB* in stem; *EIF4A-III* in dry seed; *HMGB3* in senescing leaf; *HOT5* in hypocotyl; *IDH-V* in root; *KCS9* in cotyledon; *KLCR1* in stem; *LOX2* in vegetative rosette; *LSD1* in pollen; *MT2A* in siliques; *OASA1* in cotyledon;

PAB1 in dry seed; *PDR2* in pollen; *P14GAMMA4* in pollen; *PP2AA2* in pollen; *PPA4* in stem; *ROC3* in root; *RS27A* in siliques; *SLP3* in flower bud; *SLT1* in siliques; *SPPL2* in flower; *SYP23* in dry seed; *THM1* in cotyledon; *TIM10* in root; *TUA2* in pollen; *UBP6* in dry seed; *XBAT32* in pollen; *XT1* in root; *NDL1* in pollen; *RAD5* in pollen.

Genes	ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14
RS27A	AT3G61110	823	666.4	731.3	2275	1576	2025	1488	1727.8	2417	829.38	866.81	262.1	2410.01	2162.54
SLP3	AT2G19170	6.92	18.2	27.7	57.35	70.91	219.6	84	74.43	63.66	7.86	34.55	43.46	16.09	93.03
SLT1	AT2G37570	156	169.9	101.9	104.7	117.8	137.9	145.3	95.48	108.6	199.86	151.03	59.65	247.86	130.35
SPPL2	AT1G63690	55.2	241.6	138.9	232.8	193.1	240.2	650.1	182.89	203.9	137.75	478.53	39.51	136.1	212.11
SYP23	AT4G17730	623	256.6	130.8	257.3	160	189.9	169.6	155.38	211.4	189.96	338.38	287.2	271.2	126.23
THM1	AT1G03680	422	1620	1848	45.13	1775	653.5	735.6	302.4	48.4	657.35	729.3	52.36	202.28	1319.3
TIM10	AT2G29530	92.3	94.59	126.5	428.8	196.8	300.7	183.1	293.3	479.4	120	119.78	71.93	90.58	246.18
TUA2	AT1G50010	95.7	88.28	148.8	863.2	502.4	878.5	402.5	709.7	762.2	30.04	1338.43	4830	64.45	972.01
UBP6	AT1G51710	693	371.1	268.1	486.1	357.4	415.9	491.9	377.06	487.4	503.45	309.61	182.6	629.66	374.4
XBAT32	AT5G57740	49.2	55.5	53.95	84.21	45.73	52.36	80.71	83.28	100.6	55.61	70.08	625.2	58.86	47.46
XT1	AT3G62720	52	84.96	269	711.1	92.31	112.2	113.8	269.03	563.1	27.65	80.13	645.9	144.61	91.38
NDL1	AT5G56750	143	78.51	79.63	115.6	56.51	62.56	319.2	100.15	98.11	48.3	94.68	8450	100.78	96.08
RAD5	AT5G22750	15.5	3.03	2.01	5.58	10.23	27.98	7.38	12.53	16.18	1.38	1.06	48.46	9.58	21.43

Expression of NDL1 and its PIs under Different Stresses

Expression of PIs genes and *NDL1* gene under different stress conditions at different time intervals were analysed using the eFP browser. Genes showing above two-fold

in each category was shortlisted (Figure 3-4).

Different PIs expressed differentially in root and shoot parts (Table 3). According to fold change values, *Nuclease* showed maximum fold change 50.2 and 16.8 (at 3hrs and 6 hrs) in shoot and root respectively in

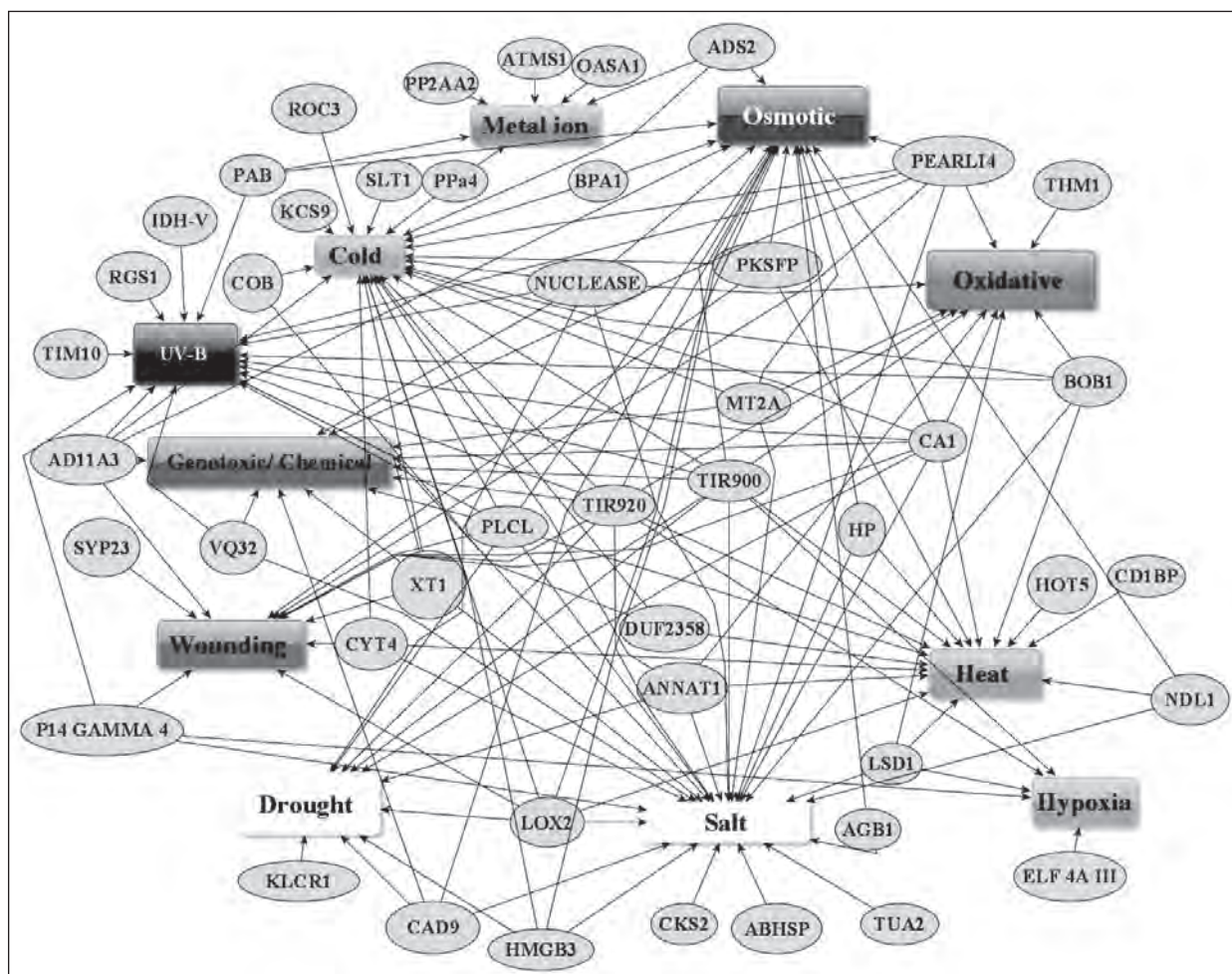


Figure 3. Genes showed above two-fold change in expression under different abiotic stresses. Elliptical boxes showing genes connected with rectangular boxes representing different stresses. Expression data collected from efp browser in TAIR. See text for more description.

Table 3, Continued.

PABI	MT2A						LSD1						CAD9						CAI						PLCL						CYT4									
	6	24	0	1	3	6	12	24	0	3	6	12	24	0	1	3	6	12	24	0	1	3	6	12	24	0	3	6	12	24	0	1	3	6	12	24	0	1	3	6
0.83	1.09	1	1.25	1.28	1.24	1.33	3.43	1	1.09	2.16	2.03	1	1.05	1.11	1.07	1	1.14	1.1	1.17	0.89	0.89	1	0.78	1.03	1.05	0.33	1	0.97	0.79	3.67										
1.84	2.52	1	1.26	1.68	1.82	1.6	2.95	1	1.06	0.71	0.5	1	1.04	2.2	1.6	1	0.91	0.94	0.69	0.37	0.11	1	0.49	0.78	0.65	0.44	1	1.22	1.34	1.24										
1.1	1.63	1	1.18	1.72	1.34	1.26	2.46	1	1.05	1.57	1.74	1	1.22	2.43	2.18	1	1.1	1.08	0.75	0.65	0.61	1	0.73	1.12	0.72	0.55	1	0.9	1.11	1.86										
1.17	1.08	1	1.07	0.91	1.28	1.04	1.11	1	0.97	1.05	1.21	1	1.47	0.98	0.68	1	0.98	0.94	0.92	0.93	1.08	1	1.18	1.35	0.74	1.11	1	1.37	1.09	1.16										
1.03	1.33	1	0.92	0.73	0.93	1.07	1.26	1	1.08	1.08	1.03	1	1.04	1.22	1.01	1	1.04	1.01	0.9	0.87	1	0.78	0.98	0.67	0.67	1	1.24	0.74	0.82											
1.09	1.31	1	0.9	0.69	1	0.8	0.97	1	1.03	1.1	1.08	1	1.02	1.2	1	1	0.9	1.12	0.94	0.95	0.93	1	0.87	1.07	0.97	0.69	1	0.92	0.74	0.9										
2.42	1.18	1	1.1	0.71	0.81	1.04	1.36	1	0.88	0.82	0.87	1	0.65	0.49	0.32	1	0.89	0.86	0.65	0.74	0.86	1	0.18	0.19	0.27	0.53	1	1.41	0.75	0.89										
1.01	1.14	1	0.8	1.06	1.18	1.04	0.83	1	0.96	0.99	0.98	1	0.55	0.71	0.84	1	0.92	0.8	0.81	0.87	1.03	1	0.89	0.72	0.61	0.81	1	2.87	1.59	0.96										
1.32	1	1	1.12	0.56	0.94	0.92	0.56	1	2.6	1	1	1	0.78	0.44	0.88	1	1.07	1.12	0.76	0.87	1	1	1.06	1.12	0.89	1.04	1	2	2.38	0.79										
0.85	0.77	1	1.1	0.64	0.71	1.33	4.86	1	0.81	1.61	2.01	1	1.21	0.73	0.96	1	0.6	0.11	1.2	1.51	8.51	1	4.43	4.27	0.42	0.42	0.99	0.94	0.93	1.49										
1.02	0.68	1	2.02	2.35	3.5	4.32	5.06	1	1.06	0.49	0.58	1	1.88	2.27	2.16	1	0.22	0.43	2.64	0.92	1.81	1	0.38	1.41	1.33	2.63	0.99	0.92	0.96	1.26										
0.59	0.66	1	1.78	1.09	0.85	3.08	3.03	1	1.11	0.61	0.71	1	1.85	0.91	0.86	1	0.15	4.12	98.2	30.3	45.8	1	1.38	2.92	3.11	2.97	0.99	0.91	0.89	1.13										
1.14	0.92	1	1.1	0.8	1.49	0.8	0.8	1	0.92	0.97	0.96	1	2.23	1.06	0.76	1	7.88	0.73	0.48	6.05	1.94	1	0.56	2.29	1.2	0.4	0.99	1.1	0.95	1.1										
1.15	1.11	1	2.21	0.88	0.97	1.08	0.87	1	0.83	0.85	0.85	1	1.5	0.81	1.3	1	0.49	1.34	8.17	9.55	6.85	1	3.22	5.01	1.36	0.54	0.99	0.85	0.9	0.94										
1.04	1.03	1	2.03	0.9	1.4	1.01	2.38	1	0.96	1.05	0.81	1	1.51	0.72	0.77	1	0.29	0.44	0.78	7.15	4.65	1	0.89	1.04	0.22	1.1	0.99	0.8	0.94	1.05										
1.04	0.87	1	1.88	0.79	1.25	1.13	1.83	1	0.79	0.91	1.06	1	1.37	0.55	0.61	1	1.04	0.45	1.39	0.54	6.79	1	1.75	3.93	0.85	1.15	0.99	0.88	0.78	1.02										
1.04	0.95	1	1.79	1.28	1.74	0.68	1.52	1	0.9	1.35	0.94	1	1.34	0.83	0.75	1	2.23	0.18	3.79	3.7	10.6	1	2.32	2.09	0.41	0.29	0.99	0.97	0.89	0.97										
1.18	1	1	0.81	0.64	1.35	1.35	0.73	1	1.64	0.76	0.88	1	1.4	0.65	0.87	1	1.19	1.11	0.86	1.42	2.77	1	0.28	3.93	0.21	0.47	0.99	2.09	3.24	1.55										

Table 3, Continued.

	VQ32									TIR920									TIR900									ANAATI									ASD2									BOBI		
	24	0	1	3	6	12	24	0	1	3	6	12	24	0	1	3	6	12	24	0	1	3	6	12	24	0	1	3	6	12	24	0	1	3	6	12	24	0	1	3								
12.1	1	1.97	1.25	0.4	0.11	0.2	1	3.62	4.53	1.62	0.06	0.18	1	0.47	1.17	0.87	0.43	0.32	1	0.75	0.52	0.89	3.12	1	2.04	8.51	26	1	1.16	1.07																		
1.66	1	1.41	0.5	0.82	0.65	0.53	1	15.1	2.8	0.94	0.75	1.02	1	3.63	2.39	1.6	2.38	5.43	1	1.71	2.3	3.1	6.37	1	0.52	0.4	0.6	1	1.01	1.57																		
2.05	1	0.93	0.5	0.64	0.48	0.57	1	1.07	0.81	1.34	1.38	0.66	1	0.39	0.67	1.03	1.95	1.67	1	2.51	3.09	3.15	5.86	1	0.71	1.12	1.84	1	0.79	0.97																		
1.34	1	1.5	0.79	0.97	1.02	1.14	1	5.15	1.23	0.87	1.45	0.81	1	1.85	1.02	1.31	2.94	1.56	1	1.55	1.27	1.34	1.22	1	0.68	0.99	1.3	1	1.12	1.1																		
1.1	1	0.84	0.81	1.66	2.13	2.38	1	0.81	1.31	1.46	1.68	3	1	0.73	0.68	1.2	2.55	3.03	1	0.75	0.84	1.31	1.46	1	0.68	0.87	1.12	1	0.89	0.97																		
1.46	1	0.91	0.82	1.31	1.23	1.19	1	0.64	1.17	1.23	1.93	1.83	1	0.52	0.75	1.18	4.97	8.95	1	0.95	0.88	0.85	1.22	1	0.73	0.83	1.22	1	1.1	0.97																		
1.25	1	3.51	2.43	1.8	1.01	1.42	1	17.1	9.93	5.09	1.46	1.74	1	10.7	11	9.15	2.97	4.53	1	0.56	0.6	1.16	1.26	1	0.45	0.96	1.06	1	0.65	2.15																		
1.77	1	1.15	1.04	1.39	1.51	1.53	1	0.95	2.56	1.66	2.35	1.73	1	0.47	2.41	2.45	4.37	2.43	1	1.4	0.99	0.97	1.39	1	0.68	0.99	0.97	1	0.83	1.04																		
0.96	1	1.02	0.88	0.93	1.03	1.09	1	0.9	0.08	0.93	0.93	1.1	1	0.51	0.35	1.4	0.89	1.01	1	0.38	0.5	1.06	1.32	1	0.9	0.7	0.73	1	12.3	9.74																		
1.99	1	0.99	0.96	1.01	0.92	0.9	1	2.97	3.38	10.7	4.66	2.53	1	2.03	1.67	6.39	6.59	6.43	1	1.08	0.98	1.1	1.5	1	2.54	4.5	7.45	1	0.91	0.93																		
1.21	1	0.67	0.76	1.16	1.38	1.11	1	1.29	0.79	1.14	1.4	1.5	1	1.73	1.16	2.24	3.27	3.21	1	1.01	0.92	1.11	1.23	1	0.63	0.54	0.59	1	0.96	0.97																		
1.21	1	1.27	2.23	3.15	2.48	1.75	1	4.09	5.61	8.97	2.01	1.53	1	1.98	3.19	5.11	3.69	3.18	1	0.64	0.41	1.04	1.41	1	0.56	0.52	0.62	1	0.98	0.68																		
1.06	1	1.19	0.82	0.99	0.91	0.88	1	1.96	0.69	1.29	0.85	1.09	1	2.3	1.07	1.93	1.02	1.29	1	1.08	0.86	1.19	1.05	1	0.91	1.05	1	1	1.12	1.08																		
0.93	1	1.52	0.95	1.23	1.2	1.22	1	1.65	0.93	1.64	1.98	1.87	1	2	1.43	2.06	2.4	1.75	1	1.09	0.73	1.04	1.26	1	0.84	0.82	0.88	1	0.95	0.94																		
0.86	1	1.15	0.94	1.12	1.13	1.14	1	1.73	0.51	0.89	1.17	1.38	1	1.6	0.71	1.56	1.58	2.07	1	1.2	0.76	0.93	0.82	1	0.76	0.85	0.72	1	0.96	0.86																		
1.16	1	0.99	0.78	1.02	1.19	0.9	1	2.38	0.63	1.03	0.99	0.85	1	2.55	1.6	1.45	1.1	1.64	1	1.29	0.96	1.12	1.09	1	0.77	0.82	0.93	1	0.94	0.91																		
1.03	1	1.33	1.04	1.42	1.22	1.25	1	1.79	0.64	1.15	0.48	1.65	1	1.66	0.86	2.22	1.66	2.66	1	1.11	0.88	1.27	1.18	1	0.79	0.84	0.95	1	0.85	0.81																		
0.93	1	0.96	0.74	1.22	1.31	1	1	1.57	0.5	0.92	0.87	1.18	1	2.67	1.1	1.29	0.67	1.08	1	0.47	0.71	0.85	0.99	1	0.88	0.64	0.73	1	4.07	5.15																		

Table 3, Continued.

ALDH11A3												DUF2358						HP						NUCLEASE						PEARL14	
6	12	24	0	1	3	6	12	24	0	1	3	6	12	24	0	1	3	6	12	24	0	1	3	6	12	24	0	1			
1.32	0.98	2.18	1	1.24	0.99	1.26	1.07	0.48	1	1.26	1.75	0.73	1	0.93	1.09	1	1.1	0.92	0.71	1	0.85	1.22	1.67	1	4.16	50.3	13.6	2.04	1.86	1	0.43
1.73	2.4	3.48	1	0.98	0.81	1.01	0.65	0.78	1	1.44	1.17	1.08	1	1.16	1.31	1	0.96	0.94	1.09	1	0.86	3.48	4.58	1	8.7	1.81	1.48	2.46	1.87	1	2.25
1.34	1.67	2.6	1	1.16	1.15	1.18	0.83	0.79	1	1.76	1.26	0.93	1	0.98	1.45	1	1.04	0.68	0.59	1	1.25	1.03	1.67	1	1.12	1.27	1.4	2.03	2.55	1	0.83
0.81	1.08	0.86	1	1.18	1.06	1.25	0.95	1.09	1	0.93	1.03	0.97	1	1.03	1.27	1	0.92	0.89	0.75	1	0.78	1.21	1.4	1	9.47	0.91	1.37	1.21	1.46	1	1.84
0.88	1.38	1.12	1	1.05	1.07	1.27	0.9	1.02	1	1.17	1.21	0.93	1	1.07	1.1	1	0.84	0.95	0.76	1	0.99	1.19	1.17	1	0.86	0.77	0.9	1.11	2.6	1	1.69
0.9	1.45	2.08	1	1.05	1.11	1.09	0.91	0.86	1	0.95	1.12	1.15	1	0.93	1.27	1	1.08	0.86	0.68	1	0.75	1.12	0.92	1	0.63	1.05	0.86	1.55	2.95	1	0.73
3.61	1.97	1.81	1	0.94	0.76	0.69	0.89	0.9	1	0.81	0.67	0.68	1	1.95	2.66	1	1.4	0.73	0.85	1	0.91	1.3	1.3	1	8.44	3.87	2.22	1.08	1.29	1	4.1
1.23	1.28	1.39	1	0.83	0.86	0.91	0.94	0.97	1	0.87	0.87	1.01	1	1.55	1.5	1	0.85	0.79	0.72	1	0.94	1.7	1.42	1	5.39	1.6	1.35	1.38	1.91	1	1.9
0.63	1.12	1.16	1	0.9	0.82	0.71	0.92	0.84	1	1.15	0.8	0.95	1	1.13	2.07	1	0.89	1.44	1.08	1	1.88	0.83	0.77	1	1.53	1.12	0.92	1.13	1.22	1	0.32
0.83	0.75	0.83	1	1.74	3.05	2.53	1.16	1.02	1	1.36	1.28	1.43	1	0.81	1.05	1	1.39	1.31	4.5	1	1.31	1.06	1.07	1	1.07	5.53	16.1	12.2	9.44	1	0.89
1.07	0.8	0.78	1	0.69	1.32	1.67	2.32	2.78	1	1.46	1.18	1.57	1	0.57	0.91	1	0.93	1.65	1.78	1	1.39	1.13	1.22	1	1.86	0.84	2.43	4.1	2.27	1	0.79
0.67	0.77	0.83	1	1.44	0.93	0.63	1.66	1.86	1	2.06	2.43	2.72	1	0.55	0.58	1	0.72	1.53	1.48	1	2.06	0.81	0.65	1	7.25	14.9	62.1	24.7	19	1	1.4
1.05	1.01	0.97	1	1.11	1.18	0.92	0.74	0.86	1	1.16	1.07	1.1	1	0.67	1.15	1	0.75	1.37	0.65	1	1.99	0.69	1.27	1	3.78	0.85	1.19	0.92	0.93	1	0.85
1.06	0.85	0.83	1	2.03	2.72	1.64	1.54	1.67	1	1.16	1.12	1.03	1	0.88	1.24	1	1.01	1.18	0.87	1	1.74	1.13	1.21	1	0.75	0.6	1.11	0.94	1.31	1	1.23
1.07	1	0.97	1	1.37	1.57	0.95	1.28	1.24	1	0.89	1.01	1.52	1	0.84	1.35	1	0.97	0.79	0.79	1	1.97	1.07	1.43	1	0.69	0.56	1.16	1.21	1.16	1	1.23
0.97	1	0.91	1	1.18	2.12	1.23	1.36	1.08	1	0.97	0.87	0.99	1	0.93	1.24	1	2.49	1.25	1.24	1	1.73	0.83	0.67	1	0.61	0.39	0.56	0.79	0.79	1	1.4
0.94	1.05	0.81	1	1.35	2.08	2.22	0.61	1.75	1	0.98	1.35	1.02	1	0.92	1.29	1	0.86	1.44	0.79	1	1.51	0.66	1.21	1	0.8	0.58	1.19	2.48	3.02	1	1.15
0.99	1.15	0.99	1	0.91	1.54	1.37	0.64	0.78	1	1.97	0.75	1.17	1	0.56	1.44	1	0.76	2.94	1.31	1	2.17	0.63	0.87	1	0.8	0.75	0.95	0.96	1.12	1	0.76

Table 3, Continued.

PEARLI4				PKSFP				BPA1		IDHV		ROC3		SYP23		TIM10					
3	6	12	24	0	1	3	12	24	0	12	24	0	3	6	0	24	0	12	0	6	12
1.33	0.81	0.92	4.94	1	1.2	1.14	2.68	3.42	1	1.36	2.52	1	0.88	0.65	1	2.37	1	0.78	1	0.84	0.96
2.68	1.01	1.22	8.8	1	1.04	1.64	2.18	2.1	1	2.33	2.9	1	1.61	1.21	1	0.94	1	1.76	1	0.77	0.84
0.44	0.65	0.46	1.71	1	1.05	1.65	1.46	1.34	1	1.34	1.47	1	0.98	0.92	1	1.08	1	1.25	1	0.73	1.05
0.62	0.64	1.12	1.6	1	1.96	1.13	1.07	0.97	1	0.97	0.93	1	1.07	1.06	1	1.07	1	1.06	1	0.73	1.02
0.63	1.09	1.64	3.34	1	1.22	0.99	1.11	1.01	1	1	1.07	1	0.96	0.96	1	1.23	1	1.28	1	0.89	1.03
0.94	1.06	1.36	5.84	1	1.07	0.95	1.06	1.05	1	0.97	0.97	1	1.01	0.92	1	0.84	1	1.33	1	0.91	1.01
5.64	2.22	1.57	5.89	1	0.99	1.55	1	0.97	1	1.2	1.14	1	2.07	2.21	1	1.51	1	0.88	1	2.47	2.34
0.98	1.04	0.86	3.21	1	2.27	1.17	0.9	1.1	1	0.98	0.92	1	1.22	0.92	1	0.8	1	0.97	1	1.24	1.16
0.22	0.82	1.06	1.34	1	1.18	2.41	0.94	0.85	1	1.06	1.04	1	1.09	0.75	1	0.9	1	1.13	1	1.06	1.08
1.15	1.15	1.98	1.59	1	0.9	1.06	2.46	2.46	1	1.11	1.27	1	0.88	0.81	1	1.06	1	0.94	1	0.82	0.82
0.69	0.78	1.2	0.78	1	2.04	1.3	1.61	1.43	1	1.48	1.53	1	1.01	0.99	1	0.74	1	1.61	1	0.72	0.43
2.78	2.93	2.76	2.15	1	1.66	1.05	1.14	0.91	1	1.12	0.88	1	0.83	0.83	1	0.85	1	0.94	1	0.42	0.35
1.08	0.95	1.07	0.89	1	1.45	1.03	0.98	0.8	1	0.99	1.04	1	1.02	1.07	1	1.09	1	1.11	1	0.89	0.99
1.16	1.02	1.25	1.25	1	1.09	0.85	0.92	0.81	1	1.04	0.96	1	0.88	0.9	1	1.01	1	1.18	1	0.95	0.84
1.26	1.07	1.22	1.06	1	1.05	1.05	1.11	1.03	1	1.01	1	1	0.98	0.93	1	0.92	1	1.22	1	0.96	0.93
0.98	0.99	1.11	1.03	1	1.05	0.85	1.02	1	1	1.03	1.06	1	0.88	0.97	1	0.96	1	1.23	1	0.88	1.01
1.06	1.15	1.69	1.39	1	0.97	0.95	1.08	1	1	0.89	0.91	1	0.99	0.95	1	0.94	1	2.08	1	0.77	0.8
0.34	1.05	1.12	1.01	1	1.2	2.56	1.04	0.9	1	0.98	0.98	1	0.84	0.95	1	1.06	1	1.65	1	0.7	1

cold stress; *TIR900* and *MT2A* (*Metallothionein 2A*) showed maximum fold change 15.07 and 5.06 (at 1 hr and 24 hrs) in shoot and root parts respectively in osmotic stress; *ANNAT1* and *CA1* (*Carbonic anhydrase I*) showed maximum fold change in salt stress 5.86 and 98.22 (at 24 hr and 6 hr) in shoot and root respectively. In the case of drought stress *NUCLEASE* and *CA1* showed maximum fold change 9.4 and 7.88 (at 1 hr) in shoot and root part; in genotoxic stress *PEARLI4* and *CA1* showed maximum fold change 3.34 and 9.55 (at 24 hrs and 12 hrs) in shoot and root part respectively. *TIR900* and *CA1* showed maximum fold change 8.75 and 7.15 (at 24 hrs and 12 hrs) in oxidative stress in shoot and root respectively; *TIR920* and *CA1* showed maximum change in the case of UV-B stress with 17.11 and 6.79 (at 1 hr and 24 hrs) fold in shoot and root part respectively. *NUCLEASE* and *CA1* showed maximum change upon wounding with 5.39 and 10.57 (1hr and 24 hrs) fold in shoot and root part. Under heat stress, *BOB1* showed maximum fold change in both shoot (12.32 at 1 hr) and root (5.15 at 3hr). Some genes showed more expression in comparison to other genes. Overall, many of the genes like *ATMS1*, *ASD2*, *CYS4*, *LOX2* showed prominent change in expression

in different stress conditions. Some of the genes for example, *PI4G4*, *HOT5*, *KCS9*, *PPA4*, *ELF4AIII*, *PLCL* have low expression but their expression changes with stress but remain much low than other genes. Whereas genes like *CKS2*, *PAB* and few others showed almost no or minimum variation in stress condition. Majority of the PIs showed upregulation in the expression levels like *CA1*, *LOX2*, *MT2A*, *CAD9*, *OASAI* under different stresses were in shoot part, while the overall expression levels in root part remained lower as compared to the shoot part. Mainly in stress condition the upregulation of genes involved in regulating amelioration of stress was seen. Major genes affected irrespective of type of stress were *CA1*, *NUCLEASE*, *TIR900*, *TIR920*, *ANNAT1*, *PEARLI4*, *BOB1* and *MT2A* etc.

Hormonal treatment expression analysis data was also retrieved from eFP browser and it was found that PIs genes like *NUCLEASE* (under auxin, different brassinosteroids treatment), *TIR900* (under ethylene treatment), *CDIBP* (under cytokinin and GA3 treatment), *ABHSF* (under ABA treatment), *XTI* (under brassinolide and deoxotyphasterol), *PLCL* (under campestanol treatment), showed maximum fold change in expression under specific hormonal treatments (Figure 4A).

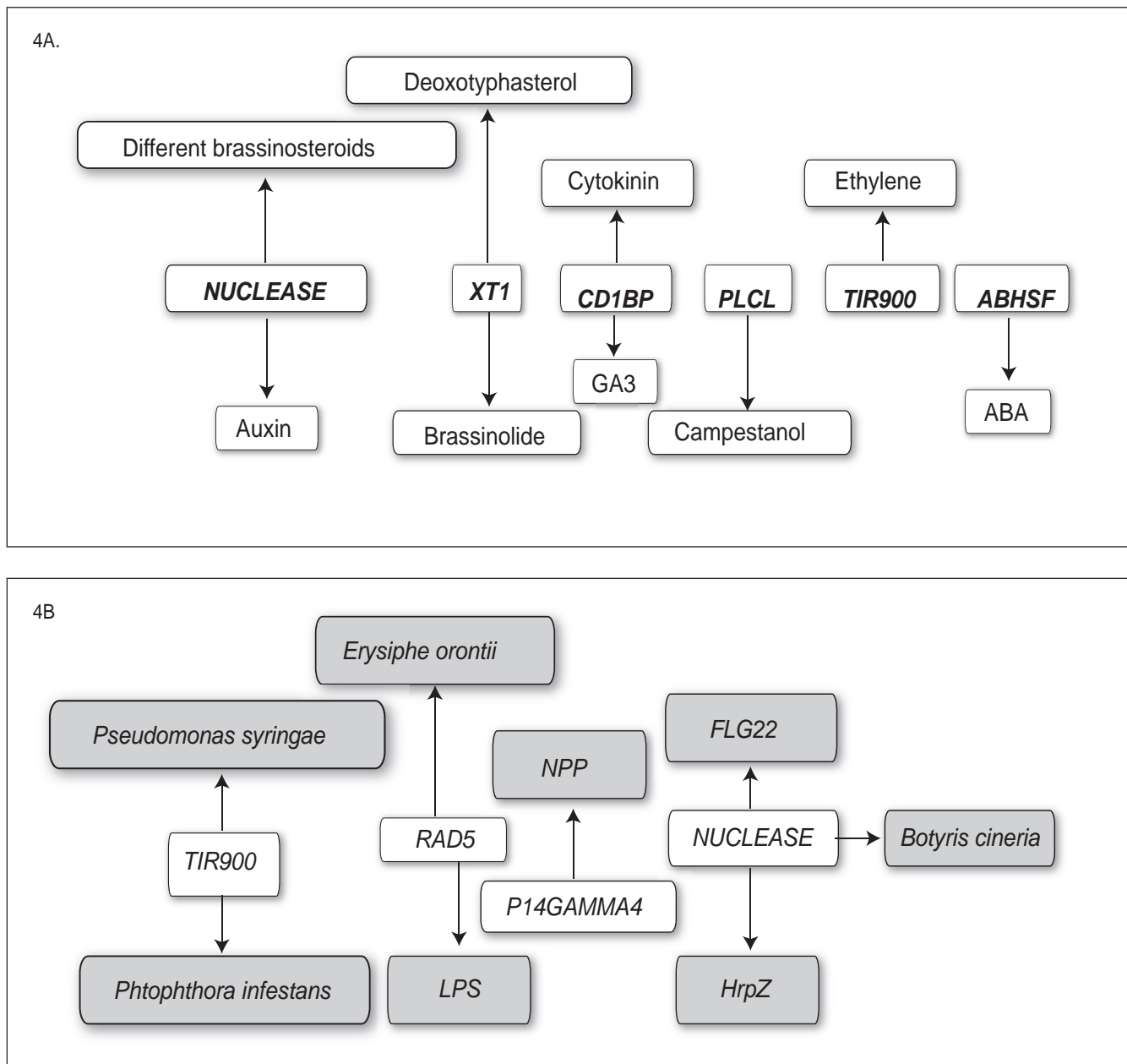


Figure 4. (A) Genes from interactome showing maximum fold change in response to various hormonal treatment. *NUCLEASE* (under auxin, different brassinosteroids treatment), *TIR900* (under ethylene treatment), *CD1BP* (under cytokinin and GA3 treatment), *ABHSF* (under ABA treatment), *XT1* (under brassinolide and deoxytyphasterol), *PLCL* (under campestanol treatment), showed maximum fold change in expression under respective treatments; **(B)** Genes from NDL1 interactome showing maximum fold change in response to different biotic stress treatments. *Botrytis cineria* caused change in expression of *NUCLEASE* by 2.66-fold, *TIR900* expression change by 3.21-fold in the case of *Pseudomonas syringae* and 6.78 in the case of *Phytophthora infestans*, *RAD5* expression change by 3.50-fold in the case of *Erysiphe orontii*. Change in expression of genes was also seen when treated with different elicitors like, *FLG22* and *HrpZ* cause the expression change by 4.82- and 5.49-fold change of *NUCLEASE*; *LPS* causes change in expression of *RAD5* by 3.76-fold; *NPP* cause change in expression of *P14GAMMA4* by 2.35-fold. Refer annexure to see the change in expression of other PI's. Data retrieved from efp browser in TAIR.

Some of the PIs also showed change in different biotic stresses (Figure 4B). For instance, treatment with *Botrytis cineria* cause maximum change in expression of *NUCLEASE* by 2.66-fold likewise, *TIR900* expression change by 3.21-fold in the case of *Pseudomonas*

syringae and 6.78 in the case of *Phytophthora infestans*, *RAD5* expression change by 3.50-fold in the case of *Erysiphe orontii*. Change in expression of genes was also seen when treated with different elicitors like, *FLG22* and *HrpZ* cause the expression change

by 4.82- and 5.49-fold change of *NUCLEASE*; LPS causes change in expression of *RAD5* by 3.76-fold; NPP cause change in expression of *P14GAMMA4* by 2.35-fold. *In planta* validation of these finding would be helpful in knowing their molecular mechanism of action.

Discussion

Recent reports on NDL proteins predict their role in abiotic stresses and propose them to be possible stress markers (Rymaszewski et al., 2017; Katiyar & Mudgil, 2019). Advancement in molecular biology combined with metabolomics, transcriptomics and proteomics approaches is useful in predicting the probable candidate genes and functions of these in plants. Present study is also similar initiative which highlights the role of NDL1 and its PIs in different stresses, hormones and expression analysis in different parts of plant.

Current work will help us in predicting the role and in planta expression pattern/levels of PIs of NDL proteins in plants. We have shortlisted these PIs on the basis of fold change expression in stress conditions which might have some role in plant to deal with stress. Our study also indicates probable involvement of NDLs during stress signaling pathway with different PIs in normal and stress conditions. We found that there are shared/common PIs between various G protein core components and NDL1 meaning that there might be cross talk or shared pathways between them during stress responses (Figure 1). Jose and Chaudhary (2020) also recently reviewed that G protein subunits share some common interactors involved in hormonal signalling in plants. These overlapping interactions indicate that these interactors might be functioning in combination or in some sequential order during signalling. Our study reveals these combinations to be explored further *in vivo* to provide detailed functions of these proteins.

Our networks analysis indicate that PIs of NDL1 are involved in diverse processes and functions. Although most of the interactors are putative and need to be further validated, our results indicate that NDL1 might be involved in processes related to growth and development, cell cycle, biotic and abiotic stress responses, hormonal response, and metabolic or biosynthesis processes etc. (Figure 2A). Around 50% of PIs showed response to various abiotic stresses, which may include heat, cold, salt, metal etc. Similarly, different categories like biotic stresses comprise stress caused by any pest or pathogen; hormonal response comprise

response to any of the plant hormones; metabolic or biosynthesis processes includes catabolic or metabolic processes. The developmental roles category comprises different processes like germination, organogenesis, morphogenesis etc.; gene expression includes those involved in the transcription process; cell cycle includes mitosis or meiosis; signalling can be inferred from name; others include any of the processes like part of some organelle structure, cellular transportation, or other cellular processes not included in above mentioned categories. In function network majority of PIs were involved in enzymatic functions (Figure 2B).

Expression analysis of NDL and its PIs under various abiotic and biotic stresses indicate that overall prominent role in dealing with abiotic and biotic stresses and suggest some suitable candidates for further analysis. *CA1* involved in various processes in plants including carbon management and stomatal movements (Engineer et al., 2014). There are several reports which signify that stomatal movements get affected by stress (Qi et al., 2018) due to various factors like ABA, ROS etc. Roles of *ANNAT1* in different stress conditions especially in salt and drought stress are well established. *ANNAT1* overexpression had shown better tolerance toward stress (Huh et al., 2010), a finding also seen in our analysis. Similarly, high expression levels of *PEARLI4* under superoxide was also reported in previous studies (Xu et al., 2017) which can be correlated here with high expression under genotoxic stress. The information regarding the roles of *NUCLEASE*, *TIR900*, *TIR920* are still limited however recent study on hypoxia stress revealed the change in expression (Lee and Bailey-Serres, 2019). Also, gene information present on online data server like NCBI assigns defence response, apoptosis, innate immune response to *TIR900* and *TIR920*. The role of *MT2A* in mediating the oxidative stress in plants is also confirmed by various studies (Zhu et al., 2009) which can be linked to its high expression under the same in our study.

Further *in planta* experiments can be planned with these results to elucidate the role and molecular mechanism of NDLs action in stress management. Functional characterization of PIs using over-expression and mutant based phenotypic and physiological analysis in various NDL's background will unveil their actual role in plant growth/development and NDL mediated abiotic stress signalling. It will help us in designing further experiments to explore this area in future for

plant stress management. Our findings also highlight more candidate genes which can be exploited in agriculture field for crop improvement after validation in *Arabidopsis*.

Acknowledgements

The research work in YM Lab is supported by grants from the DST-SERB (EMR/2016/002780), DBT (BT/PR20657/BPA/118/206/2016) and Faculty Research Programme Grant –IoE (2020-2021) from University of Delhi. A.K is supported by fellowship from University Grants Commission, New Delhi.

Declarations

Authors declare no conflicts of interest.

References

- Chakraborty, N., Singh, N., Kaur, K., & Raghuram, N. 2015. G-protein signaling components GCR1 and GPA1 mediate responses to multiple abiotic stresses in *Arabidopsis*. *Frontiers in Plant Science*, DOI:10.3389/fpls.2015.01000, 6: 1000.
- NCBI Research Coordinators, 2016. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, 44: D7-D19.
- Engineer, C.B., Ghassemian, M., Anderson, J.C., Peck, S.C., Hu, H., & Schroeder, J.I. 2014. Carbonic anhydrases, EPF2 and a novel protease mediate CO₂ control of stomatal development. *Nature*, 513: 246-250.
- Huh, S.M., Noh, E.K., Kim, H.G., Jeon, B.W., Bae, K., Hu, H.C., Kwaj, J.M. & Park, O.K. 2010. *Arabidopsis* annexins ANNAT1 and ANNAT4 interact with each other and regulate drought and salt stress responses. *Plant Cell Physiology*, 51: 1499-1514.
- Joo, J.H., Wang, S., Chen, J.G., Jones, A.M., & Fedoroff, N.V. 2005. Different signaling and cell death roles of heterotrimeric G protein alpha and beta subunits in the *Arabidopsis* oxidative stress response to ozone. *Plant Cell*, 17: 957-970.
- Jose, J., & Roy Choudhury, S. 2020. Heterotrimeric G-proteins mediated hormonal responses in plants. *Cell Signal*, DOI:10.1016/j.cellsig.2020.109799, 76: 109799.
- Katiyar, A., & Mudgil, Y. 2019. *Arabidopsis* NDL-AGB1 modules Play role in abiotic stress and hormonal responses along with their specific functions. *International Journal of Molecular Science*, DOI:10.3390/ijms20194736, 20.
- Khatri, N., & Mudgil, Y. 2015. Hypothesis: NDL proteins function in stress responses by regulating microtubule organization. *Frontiers in Plant Science*, DOI:10.3389/fpls.2015.00947, 6: 947.
- Kloppfleisch, K., Phan, N., Augustin, K., Bayne, R.S., Booker, K.S., Botella, J.R., Jones, A.M. 2011. *Arabidopsis* G-protein interactome reveals connections to cell wall carbohydrates and morphogenesis. *Molecular System Biology*, DOI:10.1038/msb.2011.66, 7: 532.
- Kovacevic, Z., & Richardson, D.R. 2006. The metastasis suppressor, NDRG-1: A new ally in the fight against cancer. *Carcinogenesis*, 27: 2355-2366.
- Lease, K.A., Wen, J., Li, J., Doke, J.T., Liscum, E., & Walker, J.C. 2001. A mutant *Arabidopsis* heterotrimeric G-protein beta subunit affects leaf, flower, and fruit development. *Plant Cell*, 13: 2631-2641.
- Lee, T. A., & Bailey-Serres, J. 2019. Integrative analysis from the epigenome to transcriptome uncovers patterns of dominant nuclear regulation during transient stress. *Plant Cell*, 31: 2573-2595.
- Liu, C., Xu, Y., Long, D., Cao, B., Hou, J., Xiang, Z., & Zhao, A. 2017. Plant G-protein β subunits positively regulate drought tolerance by elevating detoxification of ROS. *Biochemical and Biophysical Research Communications*, 491: 897-902.
- Ma, H., Yanofsky, M.F., & Meyerowitz, E.M. 1990. Molecular cloning and characterization of GPA1, a G protein alpha subunit gene from *Arabidopsis thaliana*. *Proceeding of National Academy of Science U S A*, 87: 3821-3825.
- Mason, M.G., & Botella, J.R. 2000. Completing the heterotrimer: Isolation and characterization of an *Arabidopsis thaliana* G protein gamma-subunit cDNA. *Proceeding of National Academy of Science U S A*, 97: 14784-14788.
- Melotte, V., Qu, X., Ongenaert, M., van Crielinge, W., de Bruïne, A.P., Baldwin, H.S., & van Engeland, M. 2010. The N-MYC Downstream Regulated Gene (NDRG) family: Diverse functions, multiple applications. *FASEB Journal*, 24: 4153-4166.
- Ming, C. H. E. N., Dong-bei XU, Guang-ning FANG, Er-hui WANG, Shi-qing GAO, Zhao-shi XU, Lian-cheng LI, Xiao-hong ZHANG, & Dong-hong MIN. 2015. G-protein β subunit AGB1 positively regulates salt stress tolerance in *Arabidopsis*. *Journal of Integrative Agriculture*, 14: 314-325.
- Mudgil, Y., Ghawana, S., & Jones, A.M. 2013. N-MYC Down-Regulated-Like proteins regulate meristem initiation by modulating auxin transport and MAX2 expression. *PLoS One*, 8: e77863.
- Mudgil, Y., Uhrig, J.F., Zhou, J., Temple, B., Jiang, K., & Jones, A.M. 2009. Arabidopsis N-MYC Downregulated-Like1, a positive regulator of auxin transport in a g protein-mediated pathway. *Plant Cell*, 21: 3591-3609.
- Pandey, S., Chen, J.G., Jones, A.M., & Assmann, S.M. 2006. G-protein complex mutants are hypersensitive to abscisic acid regulation of germination and post germination development. *Plant Physiology*, 141: 243-256.
- Piquemal, D., Joulia, D., Balaguer, P., Basset, A., Marti, J., & Combes, T. 1999. Differential expression of the *RTP/DRG1/NDR1* gene product in proliferating and growth arrested cells. *Biochimica et Biophysica Acta*, 1450: 364-373.
- Qi, J., Song, C.P., Wang, B., Zhou, J., Kangasjärvi, J., Zhu, J.K., & Gong, Z. 2018. Reactive oxygen species signaling and stomatal movement in plant responses to drought stress and pathogen attack. *Journal of Integrative Plant Biology*, 60: 805-826.
- Roy Choudhury, S., Marlin, M.A., & Pandey, S. 2019. The role of G β protein in controlling cell expansion via potential interaction with lipid metabolic pathways. *Plant Physiology*, 179: 1159-1175.
- Rymaszewski, W., Vile, D., Bediee, A., Dausat, M., Luchaire, N., Kamrowska, D., Hennig, J. 2017. Stress-related gene expression reflects morphophysiological responses to water deficit. *Plant Physiology*, 174: 1913-1930.
- Trusov, Y., Rookes, J.E., Tilbrook, K., Chakravorty, D., Mason,

- M.G., Anderson, D., Botella, J.R. 2007. Heterotrimeric G protein gamma subunits provide functional selectivity in G beta gamma dimer signaling in *Arabidopsis*. *Plant Cell*, 19: 1235-1250.
- Ullah, H., Chen, J.G., Temple, B., Boyes, D.C., Alonso, J.M., Davis, K.R., Jones, A.M. 2003. The beta-subunit of the *Arabidopsis* G protein negatively regulates auxin-induced cell division and affects multiple developmental processes. *Plant Cell*, 15: 393-409.
- Weiss, C.A., Garnaat, C.W., Mukai, K., Hu, Y., & Ma, H. 1994. Isolation of cDNAs encoding guanine nucleotide-binding protein beta-subunit homologues from maize (ZGB1) and *Arabidopsis* (AGB1). *Proceeding of National Academy of Science U S A*, 91: 9554-9558.
- Winter, D., Vinegar, B., Nahal, H., Ammar, R., Wilson, G.V., & Provart, N.J. 2007. An “Electronic fluorescent pictograph” Browser for exploring and analyzing large-scale biological data sets. *PLoS ONE*, 2: e718.
- Xu, J., Tran, T., Padilla Marcia, C.S., Braun, D.M., & Goggin, F.L. 2017. Superoxide-responsive gene expression in *Arabidopsis thaliana* and *Zea mays*. *Plant Physiology Biochemistry*, 117: 51-60.
- Zhang, W., He, S.Y., & Assmann, S.M. 2008. The plant innate immunity response in stomatal guard cells invokes G-protein-dependent ion channel regulation. *Plant Journal*, 56: 984-996.
- Zhu, W., Zhao, D.-X., Miao, Q., Xue, T.-T., Li, X.-Z., & Zheng, C.-C. 2009. *Arabidopsis thaliana* metallothionein, ATMT2A, mediates ROS balance during oxidative stress. *Journal of Plant Biology*, DOI:10.1111/j.1438-8677.2010.00398.x, 52: 585.