Plant Omics Journal
2(6):238-249 (2009)
ISSN: 1836-3644

Regulation of Polygalacturonase-inhibitory proteins in plants is highly dependent on stress and light responsive elements

Guguloth Mahesh Kumar[1], Praveen Mamidala[1] and *Appa Rao Podile[1]

Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, A.P-500046, India.

**Corresponding author: arpsl@uohyd.ernet.in*

Abstract

PG inhibiting proteins (PGIPs) are extracellular plant proteins capable of inhibiting fungal endoPGs. The identification of c*is*-regulatory elements is one of the major challenges in bioinformatics and integrates comparative, structural, and functional genomics. We have detected *cis*-acting upstream regulatory elements of PGIP-encoding genes, based on sequence analysis in seven different plant species at PLACE and PlantCARE. The upstream sequences of PGIPs contain different regulatory elements such as TATA box, CAAT box, ABRE box, ERE box, wound responsive elements, drought response elements etc., which help in induction of gene expression during plant growth and development, and biotic and abiotic stress. On the basis of the analysis of the regulatory elements we confirm the assigned function of PGIPs in plants like *Lycopersicon esculentum, Arabidopsis thaliana, Oryza sativa* cv.japonica, *Vitis venifera, Brassica napus, Brassica rapa* and *Medicago sativa*. We have also identified the presence of the most important elements, at a high frequency, responsive to light apart from wounding, salicylic acid, abscisic acid, fungal elicitors, ethylene in the upstream sequences across genera providing a link to the light and stress mediated signaling in plant defense responses.

Keywords: PGIP; *cis* regulatory elements; Light responsive elements; biotic and abiotic stress

Introduction

Plants, unlike mammals, lack mobile defender cells and a somatic adaptive immune system. Instead, they rely on the innate immunity of each cell and on systemic signals emanating from infection sites (Jones and Dangl, 2006). The perception and activation of innate immune responses in plants is often mediated by receptors of pathogen-associated molecular patterns such as lipopolysaacharides, peptidoglycans and flagellin in bacteria, chitin and ergosterol in fungi. Many of the recognition events occur in the plant cell wall, which is the first barrier to come into contact with the invading organisms. The majority of microorganisms need to breach this barrier to gain access to the plant tissue and produce enzymes that degrade the cell wall polymers. Among the cell wall degrading enzymes (CWDEs) produced by phytopathogenic fungi endopolygalacturonases (PG) are the most important being the first set of enzymes secreted. The action of PGs, on the cell wall matrix, is a prerequisite for further wall degradation by other CWDEs. PGs cleave the linkages between D-galacturonic acid residues in non-methylated homogalacturonan, a major component of pectin (De Lorenzo e*t al.,* 2001). In response, plants secrete inhibitors that suppress the CWDEs like PGs, ionically bound to the plant cell wall, to limit the fungal invasion by counteracting CWDEs activity.

The plant apoplast during plant–pathogen interactions is an ancient battleground that holds an intriguing range of attacking enzymes and counteracting inhibitors (Johana and Renier 2008). The molecular struggles result in positive selection for variation of residues at the interaction surface between enzymes and inhibitors. Selection on these proteins results in either replacement of outdated versions (arms race) or different isoforms and enzymes and inhibitors are maintained in the population. Initially, these inhibitors were probably

Table 1. List of PGIPS with their accession numbers analyzed within 1,000 bp in the 5′ direction of the ATG translation start site

S.No	Accession number	Name of the Plant	Name of the PGIP	Length of upstream sequence	Reference
1	AAA53547.1	*Lycopersicon esculentum*	PGIP	370	Stotz *et al*., 1994
2	AAF69827.1	*Arabidopsis thaliana*	PGIP1	1000	Park *et al*., 2000 (NCBI Direct Submission)
3	AAF69828.1	*Arabidopsis thaliana*	PGIP2	507	Park *et al*., 2000 (NCBI Direct Submission)
4	CAJ55691.1	*Oryza sativa* (Cv.Japonica)	PGIP1	1000	Janni *et al.,* 2006
5	CAJ55692.1	*Oryza sativa* (Cv.Japonica)	PGIP2	1000	Janni *et al.,* 2006
6	CAJ55693.1	*Oryza sativa* (Cv.Japonica)	PGIP3	1000	Janni *et al.,* 2006
7	CAJ55694.1	*Oryza sativa* (Cv.Japonica)	PGIP4	1000	Janni *et al.,* 2006
8	ABX46548.1	*Brassica napus*	PGIP1	1000	Hegedus *et al*., 2008
9	ABX46549.1	*Brassica napus*	PGIP2	1000	Hegedus *et al*., 2008
10	ABX46550.1	*Brassica napus*	PGIP3	1000	Hegedus *et al*., 2008
11	ABX46551.1	*Brassica napus*	PGIP5	1000	Hegedus *et al*., 2008
12	ABX46552.1	*Brassica napus*	PGIP6	783	Hegedus *et al*., 2008
13	ABX46553.1	*Brassica napus*	PGIP7	249	Hegedus *et al*., 2008
14	ABX46554.1	*Brassica napus*	PGIP8	289	Hegedus *et al*., 2008
15	ABX46555.1	*Brassica napus*	PGIP9	1000	Hegedus *et al*., 2008
16	ABX46556.1	*Brassica napus*	PGIP10	1000	Hegedus *et al*., 2008
17	ABX46557.1	*Brassica napus*	PGIP11	248	Hegedus *et al*., 2008
18	ABX46558.1	*Brassica napus*	PGIP12	781	Hegedus *et al*., 2008
19	ABX46559.1	*Brassica napus*	PGIP13	248	Hegedus *et al*., 2008
20	ABX46560.1	*Brassica napus*	PGIP14	248	Hegedus *et al*., 2008
21	ABX46561.1	*Brassica napus*	PGIP15	766	Hegedus *et al*., 2008
22	ABX46562.1	*Brassica napus*	PGIP16	783	Hegedus *et al*., 2008
23	ABX46563.1	*Brassica napus*	PGIP17	248	Hegedus *et al*., 2008
24	ACP28178.1	*Brassica rapa*	PGIP1	1000	Kim *et al*., 2009 (NCBI Direct submission)
25	ACP28176.1	*Brassica rapa*	PGIP2	1000	Kim *et al*., 2009 (NCBI Direct submission)
26	AAK14075.1	*Vitis Vrnefera*	PGIP	1000	Bezier *et al*., 2002
27	AAZ32892.1	*Medicago sativa*	PGIP	1000	Zhang *et al*., 2005 (NCBI Direct submission)

constitutively produced, but upon evolution of pathogen recognition systems the production and secretion of these proteins became inducible, becoming part of the arsenal of pathogenesis- related (PR) proteins (Johana and Renier 2008).

PGIPs are extracellular leucine-rich repeat (eLRR) proteins that recognize and inhibit fungal PGs associated with cell walls of plants (Federici *et al.,* 2001). PGIPs are, identified in several plants including raspberry, tomato, pear, apple, grape, soybean, bean, mustard and *Arabidopsis*, encoded by small gene families that are regulated by different pathways, probably minimizing pathogen interference in PGIP expression (Ferrari *et al.,* 2003; D'Ovidio *et al.,* 2004). Plants have selected the LRR-fold for their "immune" functions and recognition of non-self molecules. Several plant resistance gene products or defense related receptors display LRR motifs of the extracytoplasmic type (Dangl and Jones 2001). The PG–PGIP interaction limits the aggressive potential of PGs, favours the accumulation of elicitor-active oligogalacturonides in the apoplast and causes the activation of defense responses (De Lorenzo and Ferrari 2002). Small gene families encode PGIP isoforms that differ in affinity and specificity for PGs secreted by different pathogens (Federici *et al.,* 2001). Plants have evolved sophisticated light sensing mechanisms that regulate acclimatory and developmental processes including pathogen defence pathways (Karpinski *et al.,* 2003). Simultaneous pathogen attack and fluctuation in light intensity and quality make rapid acclimation a constant necessity (Kulheim *et al*., 2002). The interaction of phytochrome signaling with salicylic acid SA-dependent signal transduction pathway was demonstrated in darkness or in dim light with strange reduction of the defense gene expression (Genoud *et al.,* 2002).

PGIPs being eLRR proteins that recognize and inhibit fungal PGs, share conserved features with many disease resistance genes, suggesting that PGIPs may be involved in pathogen recognition/suppression. PGIPs from a range of species often show considerable homology; yet individual isoforms appear to exhibit a degree of specificity with respect to the endo PGs that they inhibit *in vitro* (Cook *et al.,* 1999; Sharrock and Labavitch 1994; Stotz *et al.,* 2000). The wide degree of functional redundancy and recognition specificity of the PGIP gene families provides higher protection and a selective advantage for the activation of defense responses (Federici *et al.,* 2001) while the expression of PGIPs is regulated by different stress-related molecules through separate signal transduction pathways. Expression of *Pv*PGIP3

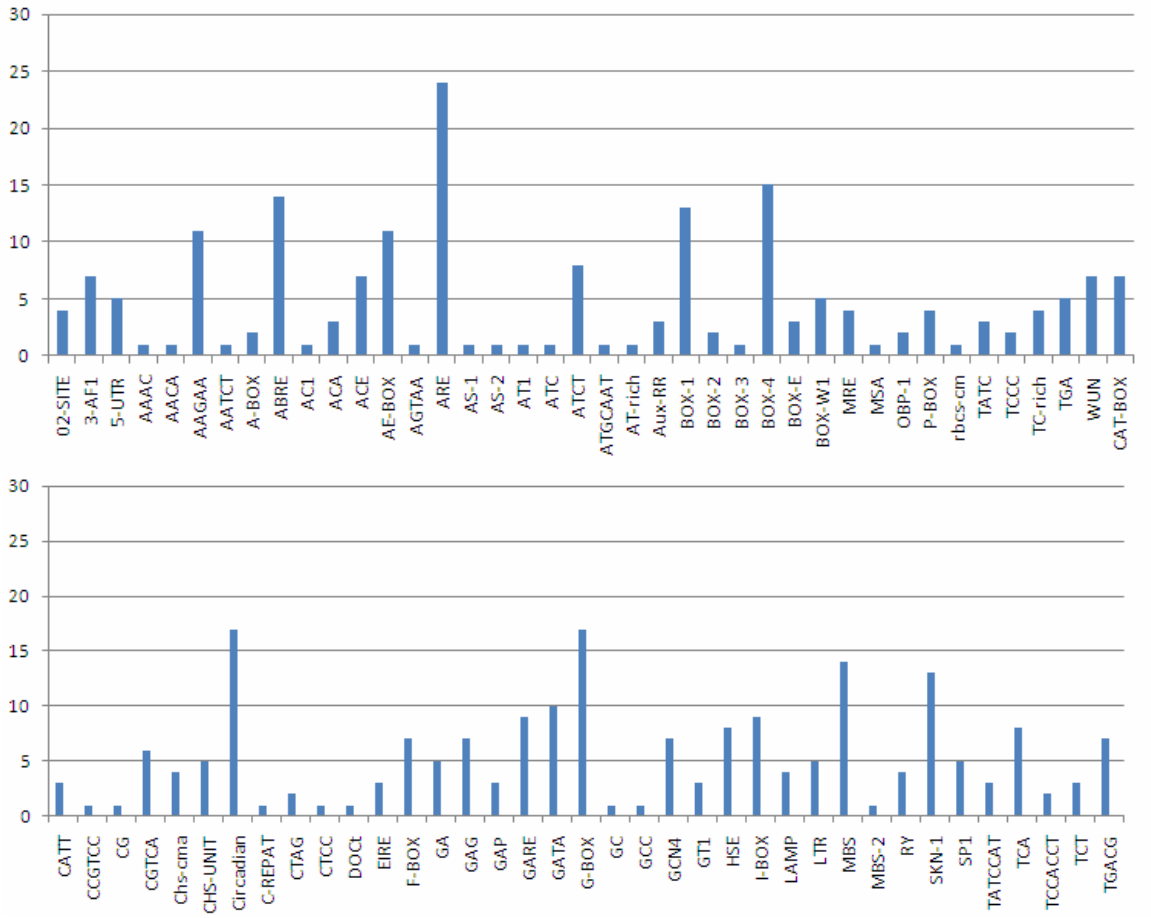


Fig 1. Frequency of *cis* element occurrence in twenty seven different PGIP upstream sequences

is induced by oligogalacturonides but not by fungal glucan or salicylic acid or wounding while expression of *Pv*PGIP4 is not altered by any of these treatments. Expression of *Pv*PGIP1 is induced by wounding only (Devoto *et al.,* 1998) whereas *Pv*PGIP2 is upregulated by oligogalacturonides, salicylic acid and wounding (D'Ovidio *et al.,* 2004). Expression of PGIPs is also regulated by various stress stimuli in *Arabidopsis*. The two genes of *Arabidopsis* are activated by wounding or *B. cinerea* infection and are responsive to different signals: *At*PGIP2 responds to jasmonate, whereas *At*PGIP1 is upregulated by oligogalacturonides but is unaffected by salicylic acid, jasmonate or ethylene (Ferrari *et al.,* 2003). Here, we report our attempts to analyse the *cis*-regulatory elements present up to 1000bp upstream of PGIP-encoding genes of *Arabidopsis thaliana, Oryza sativa* cv Japonica, *Lycopersicon esculentum, Vitis venifera, Brassica napus, Brassica rapa* and *Medicago sativa* to further understand the regulation of PGIPs in plants with special reference to defense responses.

Materials and methods

Database search and sequence analysis

PGIP-encoding gene sequences and their upstream sequences were retrieved from NCBI databases originating from *Oryza sativa* and *Arabidopsis thaliana*, V*itis vinifera*, *Medicago sativa*, *Brassica napus*, and *Brassica rapa* for analysis of the upstream sequences of PGIP genes. Sequences for *Arabidopsis thaliana*, and *O. sativa* (japonica cultivar-group) from NCBI database (http://www.ncbi.nlm.nih.gov/), *V. vinefera* from Genoscope Grape genome browser (http://www.genoscope.cns.fr/spip/), *M. sativa* from Genome browser (http://www.tigr.org/tigr-scripts/gbrowse/gbrowse/medicago_imgag/), *B. napus* and *B. rapa* from *Brassica* Genome Browser (http://www.brassica-napus.org) were retrieved and analyzed. All the upstream nucleotide sequences were retrieved and submitted to PLACE (http://www.dna.affrc.go.jp/PLACE/) (Higo *et al* 1999) and PLANTCARE

(http://bioinformatics.psb.ugent-be/webtools/plant care/html/) (Lescot *et al.,* 2002). The details of sources of the upstream sequences of PGIP encoding genes in plants are given in Table 1.

Results

We have obtained and analyzed up to 1000 bp upstream sequences of a total of 27 PGIP-encoding genes from seven representative plant species (Table 1) to understand the regulation of these genes in response to different environmental conditions with special reference to interactions with fungal pathogens. The sequences submitted to PLANTCARE and PLACE databases revealed the occurrence of a large number of different *cis* motifs in upstream sequences of PGIP genes, which have different functions in sessile plants which are usually exposed to various biotic and abiotic stress conditions.

The occurrence of *cis*-elements in all the twenty seven PGIPs is shown in Fig 1. The ARE *cis* motifs were found at high frequency (24 times) in most of the PGIPs next to circadian *cis* elements (17), G-Box (17), Box 4 (15), ABRE (14), MBS (14),SKN-1 (13) and Box 1 (13) , AE (11), GATA *cis* elements 10 and all the remaining *cis* elements occurred less than ten times in the analysed 27 sequences.

Based on the *cis*-regulatory elements, in the upstream region of the PGIP genes, the sequences are categorized into elements of Light responsiveness, component specific, elicitor specific, regulation site specific, tissue specific and elements which bind with specific transcription factors which includes DNA – DNA binding and Protein–Protein binding interactions. The functions of these predicted *cis* elements are listed in Table 2 and their arrangement on upstream and categorization of *cis* elements upstream regions is shown in Fig 2 and Fig 3. TATA box and CAAT box are common elements found in all the promoters studied which are important in initiation of transcription process.

The *Le*PGIP upstream elements indicate that the PGIP of *L. esculentum* would be expressed upon induction of wounding (W box), elicitor response (ERE), salicylic acid response (SARE), endosperm expression, auxin (ARE) and ethylene response (ER) and also to different wavelengths of light (LRE). A clustered organization of PGIP genes has been reported in *Arabidopsis thaliana*, where *At*PGIP1 and *At*PGIP2 are located 507 bp apart on chromosome 5 (Ferrari *et al.,* 2003). The upstream elements of these genes have similar boxes as *Le*PGIP1 besides drought inducibility, induction towards methyl jasmonate (MeJA) and low temperature responsive elements (LTR) with zein metabolic regulation and circadian controlling elements (CIRC).

Data obtained from Gramene for four PGIPs of *Oryza sativa* revealed that the LREs are found common in all the sequences. *Os*PGIP1 has *cis* elements like ARE, SARE, wound responsive element, meristem expression, ERE, and meristem-specific cis element TAACAAA. The *Os*PGIP2 has unique *cis*-elements such as gibberellin responsive and drought inducibility elements and share common with *Os*PGIP1 with regard to meristem expression and ABA responsive elements. *Os*PGIP3 and *Os*PGIP4 share common *cis* elements with *Os*PGIP1 and *Os*PGIP2, however *Os*PGIP4 has a unique heat stress responsive element (HSE). HSE *cis* elements respond to elevated temperatures and regulate the expression levels that minimize damage and ensure protection of cellular homeostasis.

ARE- *cis*-acting regulatory elements which are essential for the anaerobic induction and Light responsive elements (LRE) were seen in all the *Brassica napus* PGIP upstream sequences. *Bn*PGIP1 has *cis* regulatory elements for endosperm expression, abscisic acid responsiveness with a unique box for the anaerobic induction. *Bn*PGIP2 and *Bn*PGIP 3 shared most of the *cis* elements. Both have TC-rich- *cis*-acting element involved in defence and stress responsiveness, elements for endosperm expression, with LTR- *cis*-acting element involved in low-temperature responsiveness. These upstream sequences also have CGTCA- *cis*-acting regulatory element involved in the MeJA-responsiveness, As-1-Element involved in root specific expression along with an element involved in circadian control. The *Bn*PGIP2 shows special *cis* elements which have important role in induction of gene expression during biotic and abiotic stress conditions. Among them are W-BOX-induction of wounding, MBS- MYB binding site involved in drought-inducibility, HSE box, TGACG- involved in the MeJA-responsiveness, Box-W1- fungal elicitor responsive element, TCA-involved in salicylic acid responsiveness, G-Box-involved in light responsiveness, and an ARE. *Bn*PGIP5 has unique AS-2-Box- involved in shoot-specific expression and light responsiveness, GCN4-*cis*-regulatory element involved in endosperm expression, TC-rich- *cis*-acting element involved in defence and stress responsiveness, AuxRR -involved in auxin responsiveness, DRE/C-REPEAT- element involved in cold and dehydration responsiveness, HSE box, P-box- involved in gibberellin-responsive element, MBS box, TGA- auxin-responsive element, ATGCAAAT- TGAGTCA-, AE-box-, ATCT-, TCCC, GAG-, GATA-, 3-AF1 binding site-, G-Box-, and I box *cis*-acting regulatory element involved in light responsiveness.

*Bn*PGIP6 has elements for circadian control, RY-element involved in seed-specific regulation, ACE-

Table 2. Important cis regulatory elements found in upstream sequences of PGIPs with their functional description, according to their putative function retrieved from Plant CARE and PLACE

A) Component specific		
Name of the *cis* element	Sequence of *cis* element	Function assigned
ERE	ATTTCAAA	*cis*-acting regulatory element involved in ethylene-responsive element
WUN-motif	TCATTACGAA	*cis*-acting regulatory element involved in wound-responsive element
Circadian	CAANNNNATC	*cis*-acting regulatory element involved in circadian control
TCA-element	GAGAAGAATA	*cis*-acting element involved in salicylic acid responsiveness
AuxRE	TGTCTCAATAAG	part of an auxin-responsive element
AuxRR	GGTCCAT	*cis*-acting regulatory element involved in auxin responsiveness
CGTCA	CGTCA	*cis*-acting regulatory element involved in the MeJA-responsiveness
P-box	CCTTTTG	*cis*-acting regulatory element involved in gibberellin-responsive element
A-box	AATAACAAACTCC	sequence conserved in alpha-amylase
TGA-element	AACGAC	auxin-responsive element
TGACG	TGACG	*cis*-acting regulatory element involved in the MeJA-responsiveness
TATC-box	TATCCCA	*cis*-acting element involved in gibberellin-responsiveness
ABRE	CACGTG	*cis*-acting element involved in the abscisic acid responsiveness
B) Elicitor Specific		
Name of the *cis* element	Sequence of *cis* element	Function assigned
EIRE	TTCGACC	elicitor-responsive element
ELI-box3	AAACCAATT	elicitor-responsive element
Box-W1	TTGACC	fungal elicitor responsive element
C) Binding site Specific		
Name of the *cis* element	Sequence of *cis* element	Function assigned
AT-rich	ATAGAAATCAA	binding site of AT-rich DNA binding protein
CCAAT-box	CAACGG	MYBHv1 binding site
HD-Zip 3	GTAAT(G/C)ATTAC	protein binding site
Box III	CATTTACACT	protein binding site
D) Condition Specific		
Name of the *cis* element	Sequence of *cis* element	Function assigned
LTR	CCGAAA	*cis*-acting element involved in low-temperature responsiveness
GC	CCCCCG	enhancer-like element involved in anoxic specific inducibility
TC-rich	ATTCTCTAAC	*cis*-acting element involved in defence and stress responsiveness
MBS	CAACTG	MYB binding site involved in drought-inducibility
HSE	AAAAAATTTC	*cis*-acting element involved in heat stress responsiveness
ARE	TGGTTT	*cis*-acting regulatory element essential for the anaerobic induction
E) Plant Tissues Specific		
Name of the *cis* element	Sequence of *cis* element	Function assigned
HD-Zip 1	CAAT(A/T)ATTG	element involved in differentiation of the palisade mesophyll cells
CAT-box	GCCACT	*cis*-acting regulatory element related to meristem expression
OCT	CGCGGATC	*cis*-acting regulatory element related to meristem
CCGTCC-box	CCGTCC	*cis*-acting regulatory element related to meristem specific activation
GCN4	CACGGATC	*cis*-regulatory element involved in endosperm expression
Doct	TAACAAACTCCA	*cis*-acting regulatory element related to meristem specific activation
AACA	TAACAAACTCCA	involved in endosperm-specific negative expression
Skn-1	GTCAT	*cis*-acting regulatory element required for endosperm expression
F) Regulation specific		
Name of the *cis* element	Sequence of *cis* element	Function assigned
RY-element	CATGCATG	*cis*-acting regulatory element involved in seed-specific regulation
MSA	(T/C)C(T/C)AACGG(T/C)(T/C)A	*cis*-acting element involved in cell cycle regulation
O2-site	GATGATATGG	*cis*-acting regulatory element involved in zein metabolism regulation

MBS- MYB binding site involved in drought-inducibility, Box4-, AE-box-, Box I-, chs-CMA1a-, GATA-ATCT- and ACA- part of gapA involved with light response, while *Bn*PGIP7, *Bn*PGIP11, *Bn*PGIP13 and *Bn*PGIP14 have similar set of elements like ABRE, ACE- and G-Box. BnPGIP8 displayed elements like A-box- conserved in alpha-amylase, GCN4- involved in endosperm expression, CCGTCC-box related to meristem specific activation, apart from ARE, G-Box and ACE.

*Bn*PGIP9 revealed the occurrence of MBS-1- MYB binding site involved in flavonoid biosynthetic genes regulation, TATC-box in gibberellin-responsiveness, GATA-, chs-CMA1a-, Sp1-, TCT-, CATT- motif GCATTC part of a light responsive element, I-box-, G-Box-, ATCT-, BoxI, GAG- and GT1- light responsive element. GC- enhancer-like element involved in anoxic specific inducibility, ABRE, CAT-box, GARE, TGACG- & CGTCA (involved in the MeJA-responsiveness), SKN-elements for endosperm

expression, LTR, and HSE involved in heat stress responsiveness.

*Bn*PGIP10 has elements like SKN for endosperm expression, ARE, TATC-box- and GARE involved in gibberellin-responsiveness, MBS box, TC-rich-element involved in defence and stress responsiveness, LTR involved in low-temperature responsiveness, 5-UTR Py-rich stretch- Element conferring to high transcription levels, LAMP-element-, TCCC- MRE-, MRE-, AAAC-, rbcS-CMA7a- ,Box I-, 3-AF1 binding site- and Box4- part of a conserved DNA module involved in light responsiveness. DRE/C-REPEAT- element involved in cold and dehydration responsiveness, and an element involved in circadian control.

*Bn*PGIP12 has elements for circadian control, and other boxes like Box4-, AE box, BoxI, chs-CMA1a-, GATA-, ATCT and ACA- part of gapA involved with light response, RY-element involved in seed-specific regulation, ARE, MBS- MYB binding site, CAT-box related to meristem expression, whereas *Bn*PGIP15 has additional P-box- as an additional gibberellin-responsive element, Gap-box-, MRE box, GA box, LAMP element (part of a LRE), HSE, TCA- involved in salicylic acid responsiveness, GARE, and ABRE. *Bn*PGIP16 shows *cis* regulatory elements similar to *Bn*PGIP12 while *Bn*PGIP17 has ABRE and ACE elements.

*Br*PGIP1 shows elements for circadian control, GCN4- involved in endosperm expression, TCA- involved in salicylic acid responsiveness, TGACG- and CGTCA- involved in the MeJA-responsiveness, AE box-, GAG-, I-box- , 3AF1 and G-Box involved in light responsiveness, Doct- related to meristem-specific activation, ARE- essential for the anaerobic induction, GARE, A-box- sequence conserved in alpha-amylase, AACA- involved in endosperm-specific negative expression, and an ABRE. *Br*PGIP1 and *Br*PGIP3 share common *cis* elements of ARE, Box E, AE box and Box I involved in anaerobic induction and responsive elements towards light. *Br*PGIP3 has additional elements for AuxRR involved in auxin responsiveness, ERE, O2-site- involved in zein metabolism regulation, SKN- elements for endosperm expression; CATT- motif GCATTC part , Box4, Sp1, AE box, G box, MRE-MYB binding site, GT1 of a light responsive element, Box-W1, GARE, HSE, and AT-rich- binding site of AT-rich DNA binding protein.

In *Vv*PGIP elements for circadian control, HSE, TATC-box- GARE, Box-W1- fungal elicitor responsive element, LAMP-element- part, Box4, I box and GAG part of a light responsive elements were seen. *Ms*PGIP has elements involved in circadian control, MBS- MYB binding site involved in drought-inducibility, W-BOX, CAT-box-, O2-site- involved in zein metabolism regulation, ABRE, ARE, TGACG & CGTCA in the MeJA-responsiveness, GARE, TCA involved in salicylic acid responsiveness. ACE-, Box4-, 3-AF1 binding site, G-Box-, TCT-, Box-II- involved in light responsiveness are seen in upstream elements.

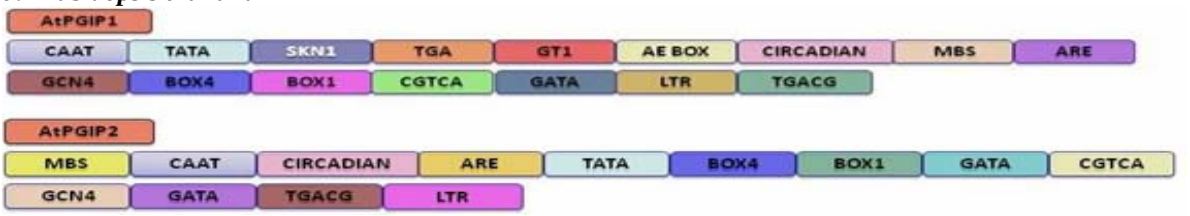
Discussion

PGIP is a constitutive protein; the amount of PGIP gene transcripts varies at different stages of maturity and at different distances from the diseased region (De Lorenzo, *et al.,* 2001). PGIPs are widely studied and several transgenic plants harboring PGIPs are well documented. PGIPs have been shown to limit fungal invasion in plants following inoculation with *Botrytis cinerea*, tomato and grapevine plants overexpressing a PGIP gene from pear (Powell *et al.,* 2000; Aguero *etal.,* 2005), *Arabidopsis* plants over expressing two endogenous PGIP genes, *At*PGIP1 and *At*PGIP2, separately (Ferrari *et al.,* 2003), and tobacco plants overexpressing the bean *Pv*PGIP2 developed smaller lesions than in wildtype plants (Manfredini *et al*., 2005). *Arabidopsis* plants with antisense expression of *At*PGIP1 show reduced inhibitory activity in response to abiotic and biotic stimuli and are more susceptible to *B. cinerea* infection, suggesting that PGIPs play a role in the innate immunity of *Arabidopsis* and contribute to its basal resistance against fungi. Expression of *Pv*PGIP3 is induced by oligogalacturonides but not by fungal glucan or salicylic acid or wounding; expression of *Pv*PGIP4 is not altered by any of these treatments. Expression of *Pv*PGIP1 is induced by wounding only (Devoto *et al* 1998), whereas *Pv*PGIP2 is upregulated by oligogalacturonides, salicylic acid and wounding (D'Ovidio *et al.,* 2004). Expression of PGIPs is also regulated by various stress stimuli in *Arabidopsis*. The two genes of *Arabidopsis* are activated by wounding or *B. cinerea* infection and are responsive to different signals. *At*PGIP2 responds to jasmonate, whereas *At*PGIP1 is upregulated by oligogalacturonide elictors but is unaffected by salicylic acid, jasmonate or ethylene (Ferrari *et al.,* 2003).

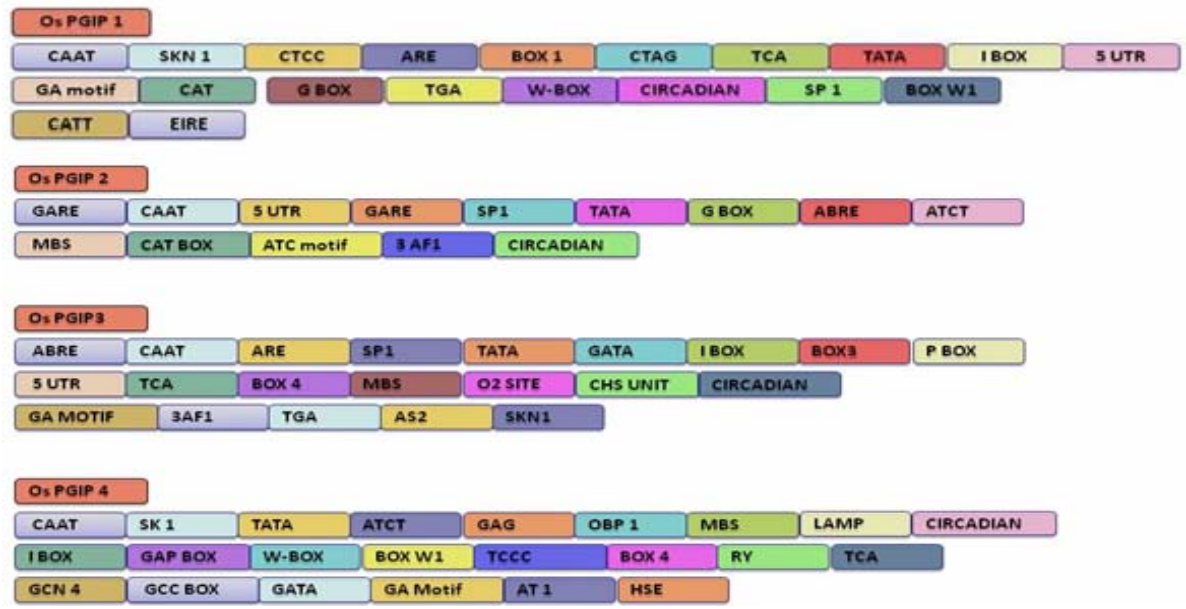
Light is a predominant factor in the control of various life processes such as growth, development, and stress responses in plants. Many biotic stress responses in plants are specifically adjusted by light conditions. However, the molecular mechanisms for plant defense against pathogen infection have only recently been linked to the light-sensing network and to the oxygen evolving complex in Photosystem II (PSII) (Mullineaux *etal* 2000 **;** Abbink *etal.,* 2002; Genoud *et al*., 2002). The amount of absorbed light energy by plants is used for photosynthetic

Fig 2. Organization of cis regulatory elements and their variability among different polygalacturonase inhibitor proteins analyzed using PLACE and PLANTCARE tools respectively. Using PLANTCARE software we found several light responsive elements.

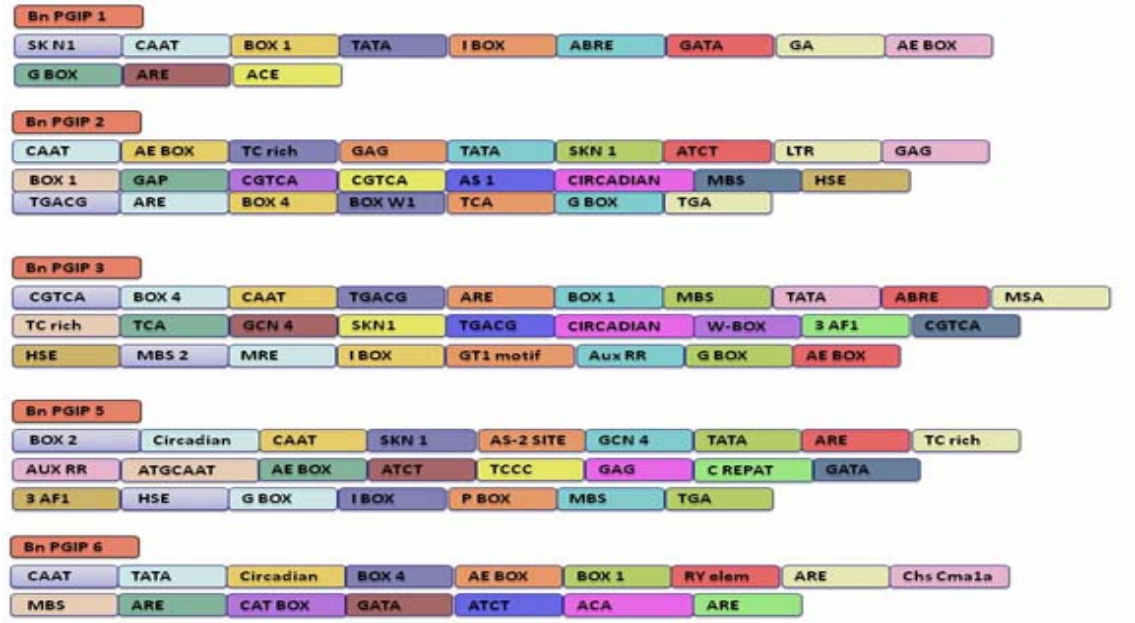
a. ***Arabidopsis thaliana***



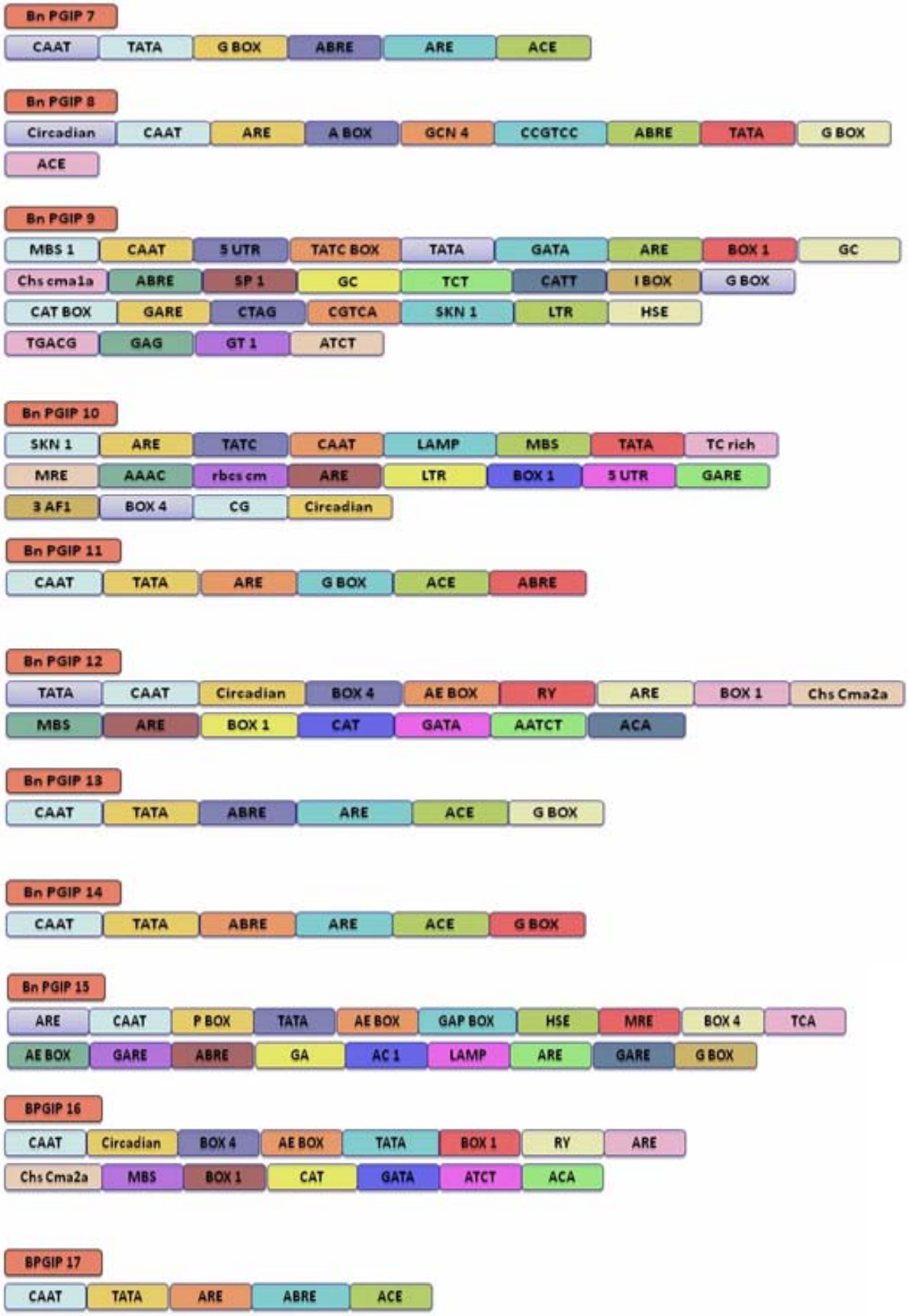
b. ***Oryza sativa*** **(cv Japonica)**



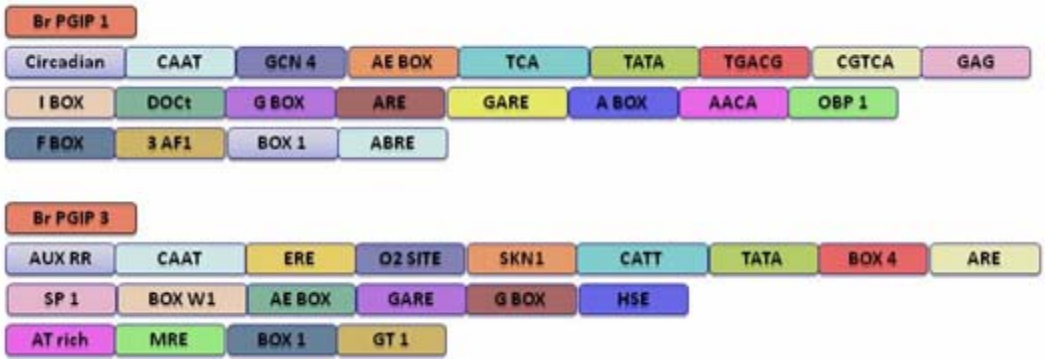
c. ***Brassica napus***



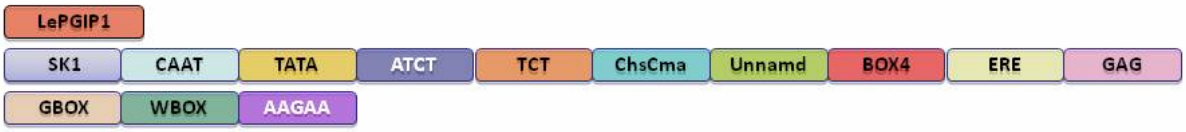
***Brassica napus* Continued**



d. ***Brassica rapa***

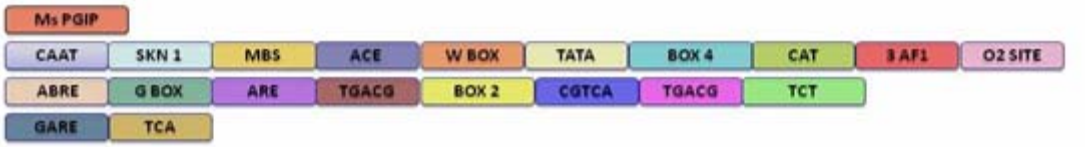


e. ***Lycopersicon esculentum***



f. ***Vitis venifera***

g. ***Medicago sativa***



metabolism and the remaining energy called excess excitation energy have several major functions such as optimization of energy status, minimization of reactive oxygen species (ROS) and as a source of information about seasonal changes (Karpinski *et al.,* 2003). During the infection of Arabidopsis leaves by an incompatible pathogen, the specific gene for gene interaction induces an array of defence responses which includes a burst of ROS (Dangl and Jones 2001). ROS contributes cell death, interfere directly with pathogen or it can act as a messenger to induce systemic acquired resistance in distant parts of the plant (Kombrink and Schmelzer 2001; Karpinska *etal.,* 2001). In the current studies we found that all the upstream sequences of 27 PGIPs have light responsive *cis* elements which could have some role in defence mechanism and control over expression of PGIP during fungal attack. These *cis*-acting elements function as molecular switches in response to environmental stress signals due to biotic and abiotic stress on plants. The W boxes are a major class of *cis* -acting elements responsible for the pathogen. W1 box {(T)TGAC(C/T)} is an important binding site for WRKY family transcription factors and has important role in transcriptional activation by auxin, SA and light (Sawant *etal.,* 2005; Rushton *etal.,* 2002). The frequency of occurrence of W box in the PGIP upstream elements (Table 2) is high next to light responsive elements. Binding sites for WRKY (W box) or AP2/ERF (GCC-like box) transcription factors can be sufficient to confer pathogen inducibility on a promoter, which represent two of the three largest families of plant-specific transcription factors (Riechmann and Ratcliffe 2000).

Plants when exposed to biotic stress, from the stage of recognition to confinement or death of the pathogen, many defense related genes would be expressed via signalling pathway which is usually carried out by signalling molecules such as SA, JA, Ethylene, ABA, hydrogen peroxide and nitric oxide (Ape and Hirt 2004; Gfeller and Farmer 2004; Durrant and Dong 2004; Mittler *etal*., 2004; Delledonne 2005; Lorenzo and Solano 2005; Torres and Dangl 2005; van Loon *etal.,* 2006).

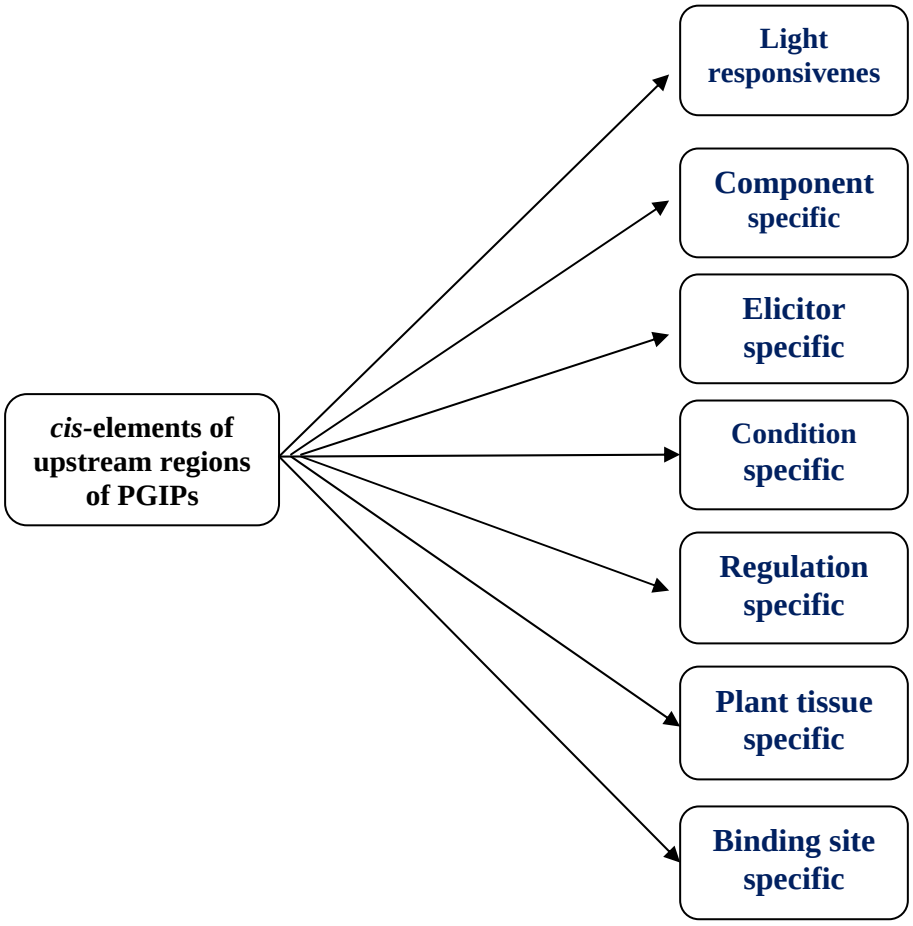


Fig 3. Categorization of cis-elements of upstream region of PGIPs based on the function assigned by Plant CARE and PLACE

SA and JA are the most recognized signals mediating transcriptional activation of pathogen related proteins (Dong 1998). The analysis of *cis* elements of various PGIPs revealed regulation by salicylic acid, jasmonic acid, ethylene production and in response to production of Absiscisc acid. ABRE is a major *cis*-acting regulatory element which has important role in adapting vegetative tissues to abiotic stresses such as drought and high salinity, as well as in seed maturation and dormancy (Shinozaki *etal.,* 2003). An 8-nucleotide ERE (ATTTCAAA) was seen in *Le*PGIP1 and *Bn*PGIP12. In the promoters of various genes that are ethylene-inducible contain EREs. Ethylene is an endogenous hormone regulating many plant processes from seed germination to plant senescence and acts as a stress hormone during adverse biotic and abiotic conditions (Bleecker and Kende 2000).

The sequence motif TAACAAA (Table 2) appears to play a central role in GA action because mutation of it caused a large decrease in GA-driven gene expression. Functional analysis of alpha amylase promoter sequences revealed that TAACAAA box is also the likely site of ABA action in repressing GA promotion of gene expression (Gubler and Jacobsen 1992). The interaction between phytohormones, particularly between gibberellic acid (GA) and abscisic acid (ABA), is an important factor controlling the transition from embryogenesis to germination in seeds. GA and ABA are antagonistic in nature i.e., GA promotes seed germination and ABA promotes seed dormancy which are antithetical phenomena. These interactions favor the seeds to germinate in favorable conditions and repress the germination process during unavoidable circum-stances. During germination embryo secretes GA

which promotes the production of hydrolytic enzymes (Yazaki *etal.,* 2003).

The *cis*-elements presented in Table 1 are known to perform different functions in plant growth and development and regulation of gene expression during biotic and abiotic stress conditions and an interesting link between the light and stress responses. However, molecular details of how these motifs interact to bring out combinatorial regulation are largely not clear**.** Hopefully further studies on these *cis*-elements will shed a new light on preparation of synthetic promoters using different motifs individually or in combinations for regulating the expression of PGIPs either for disease resistance or for developmental process.

Acknowledgements

This work was supported by Council for Scientific and Industrial Research (CSIR), New Delhi in the form of Senior Research Fellowship to GMK and University Grants Commission (UGC), New Delhi, in the form of Kothari Post Doctoral Fellowship to PM. We thank UGC-CAS, DBT-CREBB programmes of School of Life sciences and DST-FIST program of the Department of Plant Sciences, University of Hyderabad for the infrastructure support.

References

Abbink TEM, Peart JR, Mos TNM, Baulcombe DC, Bol JF, Linthorst, HJM (2002) Silencing of a gene encoding a protein component of the oxygen-evolving complex of photosystem II enhances virus replication in plants. Virology 295:307-319

Aguero CB, Uratsu SL, Greve C, Powell ALT, Labavitch JM, Meredith CP, Dandekar AM (2005) Evaluation of tolerance to Pierce's disease and Botrytis in transgenic plants of *Vitis vinifera* L. expressing the pear PGIP gene, Mol Plant Pathol 6: 43–51

Ape K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol. 2004; 55: 373–399

Bezier,A., Lambert,B. and Baillieul,F. (2002) Study of defense-related gene expression in grapevine leaves and berries infected with Botrytis cinerea Eur J Plant Pathol 108 (2), 111-120

Bleecker AB, Kende H. (2000) Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16: 1–18

Cook BJ, Clay RP, Bergmann CW, Albersheim P, Darvill AG (1999) Fungal polygalacturonases exhibit different substrate degradation patterns and differ in their susceptibilities to polygalacturonase- inhibiting proteins. Mol Plant-Microbe Interact. 12:703-711.

Dangl JL, Jones JDG (2001) Plant pathogens and integrated defence responses to infection**.** Nature 411: 826–833

De Lorenzo G, D'Ovidio R, Cervone F (2001) The role of polygalacturonase- inhibiting proteins (PGIP) in defense against pathogenic fungi. Annu Rev Phytopathol 39: 313–335

De Lorenzo G, Ferrari S. (2002) Polygalacturonase-inhibiting proteins in defense against phytopathogenic fungi. Current Opinion in Plant Biology 5: 295–299.

Delledonne M (2005) NO news is good news for plants. Curr Opin Plant Biol. 8: 390–396

Devoto A, Leckie F, Lupotto E, Cervone F, De Lorenzo G (1998) The promoter of a gene encoding a polygalacturonase-inhibiting protein of *Phaseolus vulgaris* L. is activated by wounding but not by elicitors or pathogen infection Planta 205: 165-174

Dong X (1998) SA, JA, ethylene, and disease resistance in plants. Current Opinion in Plant Biology 1: 316–323

D'Ovidio R, Raiola A, Capodicasa C, Devoto A, Pontiggia D, Roberti S, Galletti R, Conti E, O'Sullivan D, De Lorenzo G (2004) Characterization of the complex locus of bean encoding polygalacturonase-inhibiting proteins reveals subfunctionalization for defense against fungi and insects. Plant Physiol**.** 135: 2424–2435

Durrant WE, Dong X (2004) Systemic acquired resistance. Annu Rev Phytopathol 42:185–209

Federici L, Caprari C, Mattei B, Savino C, Di Matteo A, De Lorenzo G, Cervone F, Tsernoglou D (2001) Structural requirements of endopolygalacturonase for the interaction with PGIP (Polygalacturonase-inhibiting protein). Proceedings in National Academy of Sciences USA. 98 (23): 13425–13430

Ferrari S, Vairo D, Ausubel FM, Cervone F, and de Lorenzo G. (2003) Tandemly Duplicated Arabidopsis Genes That Encode Polygalacturonase-Inhibiting Proteins Are Regulated Coordinately by Different Signal Transduction Pathways in Response to Fungal Infection. The Plant Cell 15: 93–106

Genoud T, Buchala AJ, Chua NH, Traux MJP (2002) Phytochrome signalling modulates the SA-perceptive pathway in Arabidopsis. Plant J 31:87-95

Gfeller A, Farmer EE (2004) Keeping the leaves green above us. Science 306: 1515–1516.

Gubler F, Jacobsen JV **(**1992) Gibberellin-responsive elements in the promoter of a barley high-pI a-amylase gene. Plant Cell 4**:** 1435–1441

Hegedus DD, Li R, Buchwaldt L, Parkin I, Whitwill S, Coutu C, Bekkaoui D and Rimmer SR. (2008) *Brassica napus* possesses an expanded set of Polygalacturonase inhibitor protein genes that are differentially regulated in response to *Sclerotinia sclerotiorum* infection, wounding and defense hormone treatment Planta 228 (2): 241-253

Higo K, Ugawa Y, Iwamoto M, Korenaga T (1999) Plant cis-acting regulatory DNA elements (PLACE) database. Nucleic Acids Research 27(1):297-300

Janni M, Di Giovanni M, Roberti S, Capodicasa C, D'Ovidio R (2006) Characterization of expressed PGIP genes in rice and wheat reveals similar extent of sequence variation to dicot PGIPs and identifies an active PGIP lacking an entire LRR repeat. Theor Appl Genet 113 (7): 1233-1245

Johana CM, Renier ALVH (2008) Enzyme-inhibitor interactions at the plant-pathogen interface. Current Opinion in Plant Biology 11: 380-388

Jones JDG, Dangl JL (2006) The Plant Immune System. Nature 444: 323-329

Karpinska B, Karlsson M, Schinkel H, Streller S, Suss KH, Melzer M, Wingsle G (2001) A novel superoxide dismutase with a high isoelectric point in higher plants. Expression, regulation, and protein localization. Plant Physiol 126:1668-1677

Karpinski S, Gabrysy H, Mateo A, Karpinska B, Mullineauxz PM (2003) Light perception in plant disease defence signaling Current Opinion in Plant Biology 6:390–396

Kombrink E, Schmelzer E. (2001) The hypersensitive response and its role in local and systemic disease resistance. Eur J Plant Pathol. 107:69-78

Kulheim C, Agren J, Jansson S (2002) Rapid regulation of light harvesting and plant fitness in the field. Science 297:91-93

Lescot M, Dehais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouze P, Rombauts S (2002) Plant CARE, a database of plant *cis*-acting regulatory elements and a portal to tools for the *in silico* analysis of promoter sequences. Nucleic Acids Research 30: 325-327

Lorenzo O, Solano R (2005) Molecular players regulating the jasmonate signaling network. Curr Opin Plant Biol. 8: 532–540

Manfredini C, Sicilia F, Ferrari S, Pontiggia D, Salvi G, Caprari C, Lorito M, De Lorenzo D (2005) Polygalacturonase-inhibiting protein 2 of *Phaseolus vulgaris* inhibits BcPG1, a polygalacturonase of *Botrytis cinerea* important for pathogenicity, and protects transgenic plants from infection. Physiol. Mol Plant Pathol 67: 108-115

Mittler R, Vanderauwera S, Gollery M, van Breusegem F (2004) Reactive oxygen gene network of plants. Trends in Plant Science 9: 490–498

Mullineaux P, Ball L, Escobar C, Karpinska B, Creissen G, Karpinski S (2000) Are diverse signaling pathways integrated in the regulation of Arabidopsis antioxidant defence gene expression in response to excess excitation energy. Phil Trans R Soc Lond B. 355:1531-1540.

Powell ALT, Kan J, Have A, Visser J, Greve C, Bennett AB, Labavitch JM (2000) Transgenic Expression of Pear PGIP in Tomato Limits Fungal Colonization. MPMI 13 (9): 942–950

Riechmann JL, Ratcliffe OJ (2000) A genomic perspective on plant transcription factors. Current Opinion Plant Biol 3: 423–434

Rushton PJ, Reinstadler A, Lipka V, Lippok B, Somssich EI (2002) Synthetic plant promoters containing defined regulatory elements provide novel insights into pathogen- and wound-induced signaling. Plant Cell 14:749-762

Sawant SV, Kiran K, Mehrotra R, Chaturvedi CP, Ansari SA, Singh P, Lodhi, N, Tuli R (2005) A variety of synergistic and antagonistic interactions mediated by *cis*-acting DNA motifs regulate gene expression in plant cells and modulate stability of the transcription complex formed on a basal promoter. Journal of Experimental Botany. 56 (419): 2345-2353

Sharrock KR, Labavitch JM (1994) Polygalacturonase inhibitors of Bartlett pear fruits: differential effects on *Botrytis cinerea* polygalacturonase isozymes, and influence on products of fungal hydrolysis of pear cell walls and on ethylene induction in cell culture. Physiol Mol Plant Pathol 45:305-319

Shinozaki K, Shinozaki Y, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses, Curr Opin Plant Biol 6:410–417

Stotz HU, Bishop JG, Bergmann CW, Koch M, Albersheim P, Darvill AG, Labavitch JM (2000). Identification of target amino acids that affect interactions of fungal polygalacturonases and their plant inhibitors. Physiol. Mol. Plant Pathol 56:117-139.

Stotz, HU, Contos JJ, Powell AL, Bennett AB, Labavitch,J.M. (1994) Structure and expression of an inhibitor of fungal polygalacturonases from tomato Plant Mol. Biol. 25 (4), 607-617

Torres MA, Dangl JL (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. Curr. Opin. Plant Biol. 8: 397–403

van Loon LC, Geraats BPJ, Linthorst HJM (2006) Ethylene as a modulator of disease resistance in plants. Trends Plant Sci. 11: 184–191.

Yazaki J, Kishimoto N, Nagata Y, Ishikawa M, Fujii F, Hashimoto A (2003) Genomics approach to abscisic acid- and gibberellin-responsive genes in rice, DNA Research 10: 249–261.