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IN SILICO PROMOTER CHARACTERIZATION OF PR GENES AND EXPRESSION ANALYSIS IN TRANSGENIC TOMATO PLANTS EXPRESSING *OsRGLP1*

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Abstract

Transgenic approaches have shown a huge potential in crop improvement against biotic and abiotic stresses. The regulated expression of stress induced genes provides insights about the underlying molecular mechanisms. In this study, we analyzed the expression of Pathogenesis related proteins in healthy transgenic tomato plants over expressing an important stress responsive gene (*OsRGLP1*). The *OsRGLP1* was constitutively expressed in leaves and other parts of transgenic plants. We observed that *OsRGLP1* brought about an increase in the expression of tomato PR2 and PR5 genes while no significant difference was observed in the expression of tomato PR1 gene in healthy transgenic tomato plants when compared with control. The promoter analysis of all three PR genes indicated that many conserved cis acting regulatory elements (CARES) were found in the promoters of all 3 PR genes analyzed. Furthermore, some CARES were common between all three PR genes and the promoter of *OsRGLP1.* This could possibly suggest that similar transcription factors are involved in the expression and regulation of all 4 genes. These results can improve our understanding of the regulation and expression of tomato PR1, PR2 and PR5 which can be geared towards improving the overall immunity of tomato plant to stress factors in relation with GLP genes.

Key words: Pathogenesis related proteins, *OsRGLP1*, *Solanum lycopersicum,* In silico Promoter analysis.

Introduction

Just like animals and other living organisms, plants also have a defence mechanism. When plants are under attack of pathogens, a large amount of proteins usually amass, and these are referred to as Pathogenesis related proteins (Ullah *et al*., 2018). They are also known as plants defence proteins and belong to a large super-family of proteins associated with stress related and pathological conditions (Agarwal & Agarwal, 2014). These proteins have been observed in tomato, soybeans, chickpea, rice, wheat, maize, pepper and many other plants (Sudisha *et al*., 2011). Classification has been done based on different properties such as their serological relationship, amino acid composition, primary structure, enzymatic and biological activities and to this end, there are 17 known PR families (PR1 to PR17) (Ramos *et al*., 2015). Amongst these classifications, the most common classification is based on the biological activity associated with induced defence proteins (Sudisha *et al*., 2011). In plants such as tomatoes and tobacco, so far, about 11 PR proteins are known and categorized (PR1 to PR11). Related studies have shown that they play other important roles besides acting as plant defence against pathogens (Ramos *et al*., 2015). Goñi *et al.*, 2010 reported that these proteins play a significant role in fruit physiology. They found that there was an increase in expressions of PR2 and PR3 proteins in ripe fruits of *Annona cherimola Mill*. Several PR proteins detected from the intracellular and extracellular spaces of plants such as wheat, maize and sorghum possessed anti-microbial activity (Kim *et al*., 2014). Despite how extensively PR proteins have been studied in plants, their association or interaction with other proteins remains occluded. This information could be useful in determining how the expression of one protein may affect the other.

OsRGLP1 is a germin like protein (GLP) that originates from rice and characterized by its heterogeneous expression in tobacco (Yasmin *et al*., 2015). GLP's are broadly known as stress responsive proteins that possess SOD and/or OxO activity and are involved in ROS balance in the cell. The overexpression of *OsRGLP1* in transgenic tobacco plants indicated the absence of oxalate oxidase activity in roots, stems and leaves of the plant whereas significantly higher SOD activity was observed (Yasmin *et al*., 2015). In a recent research (Majeed *et al*., 2018) it is illustrated that the product of this gene is probably a heat stable iron like SOD. Additionally, they predicted that these proteins may possibly play a role in stress conditions that produces H_2O_2 and thus they concluded that it could be a more advantageous candidate gene for the improvement of crops through genetic engineering.

In this study, we tried to explore the expression dynamics of tomato PR1, PR2 and PR5 genes in transgenic tomato plants overexpressing *OsRGLP1*. No study has reported the association of GLPs with PR proteins previously. Furthermore, since limited information exist on the promoter architecture of tomato PR1, PR2 and PR5, we carried out a comparative in silico analysis of the cis acting regulatory elements (CARES) in the promoter regions of these PR genes. Interestingly, we found the *OsRGLP1*-induced enhanced expression of PR 2 and PR5 genes in leaves of the transgenic plants as compared with control. The promoter analysis of the PR and *OsRGLP1* genes also revealed the presence of common elements which may elucidate the existence of comparable underlying regulatory mechanisms.

Materials and Methods

Transgenic tomato plants: Healthy transgenic tomato plants previously established in Biochemistry, Molecular

Biology and Biotechnology lab at the Department of Biosciences, COMSATS University Islamabad, Islamabad campus were used.

Confirmation of transformed tomato plants: Using the cetyl dimethylammonium bromide (CTAB) method (Richards, 1997), genomic DNA was extracted from the leaves of healthy control and transgenic tomato plants. To reconfirm the presence of the transgene, PCR was done using the primers RGLP1P-F1:5' CCCGGGACCAACG AAAAGATTGAACA 3' and RGLP1P-R1:5' CCCGGG CATTTGTCCATGGAGAGGAT 3'.

Expression analysis of pathogenesis related genes

Total RNA isolation: Using mini-column purification kit (Thermo Scientific, USA), RNA was isolated from the leaves of transgenic as well as control tomato plants according to the user's protocol. To ensure the purity of RNA, it was given a DNase treatment. RNA pellet gotten was then air dried and dissolved in DEPC treated water and thereafter stored at -80˚C.

RNA quantification: In order to determine the quality of the extracted RNA, analysis was done on 1.2 % agarose gel and also quantified using Nanodrop (Colibri Microvolume Spectrometer-Titertek-Berthold).

Synthesis of cDNA: RevertAid First Strand cDNA Synthesis Kit #K1621, K1622 (Thermo Scientific, USA) was used to synthesize cDNA from RNA following the user's protocol. After synthesis, it was stored at -20˚C for preservation.

RT-PCR analysis: The expression pattern of PR proteins was determined using real time polymerase chain reaction (Real Time Applied Type Biosystem Step1). This was done using specific primers to amplify the cDNA in order to determine their expression levels. Specific primers used are; PR1F: 5'-GGATCGGACAACGTCCTTAC-3' and PR1R: 5'-GCAACATCAAAAGGGAAATAAT-3' (Gen bank accession number: Y08804), PR2F: 5'-AAGTAT ATAGCTGTTGGTAATGAA3' and PR2R: 5'-ATTCTC ATCAAACATGGCGAA-3' (Gen bank accession number: NM001247229), PR5F: 5'- GCAAC AACTGTCCATA CACC-3' and PR5R: 5'-AGACTCC ACCACAATCACC-3' (Gen bank accession number: NM001247422). Amplification was done using 1 µL of cDNA with a concentration of 100ng/ µL, 2 µL (F + R) of primer mix with a concentration of 10pM/ µL. The reporter used was 5 µL of the Maxima SYBR Green/ROX qPCR Master Mix (2X)(Thermo Scientific, USA). The reaction mixture had a final volume of 10 µl. The PCR conditions used were: Denaturation at 95°C for 10 s, annealing at 54°C for 45 s, extension at 72°C for 15 s for 40 cycles and final extension at 72°C for 20 minutes. Actin (GenBank accession number: NM_001330119.1) F: 5'-GGAATGG TCAAGGCTGGGTT-3'; R: 5'-CCACT GGCATACAG TGAGAGT-3') was used as the internal reference to normalize the expression data. Relative expression levels was calculated using the $2^{-\Delta\Delta Ct}$ (cycle threshold) method (Livak and Schmittgen, 2001).

Statitical analysis

Three technical replicates were used and single factor Analysis of variance (ANOVA) (p<0.05) was applied to check the differences in PR genes expression between the transgenic and wild type tomato plant samples. This was followed by Least Significant Difference (LSD) to determine where exactly those differences were significant.

Bioinformatics analysis to elucidate the promoter architecture

Extracting promoter sequences of pathogenesis related protein and *OsRGLP1*: Sequences of tomato pathogenesis related proteins analyzed in this study were accessed on NCBI. They are; PR1 gene (GenBank Accession no. Y08804), PR2 gene (GenBank Accession no. NM001247229) and PR5 gene (GenBank Accession no. NM001247422), *OsRGLP1* (GenBank Accession no. EU742684). 1.5kbp upstream of the transcription start site (TSS) was then extracted in the case of PR2 and PR5, while 1.228kbp was extracted for *OsRGLP1*. For PR1 gene, only about 400bp exist upstream of the TSS of this gene and this was used in its entirety.

Promoter analysis tool: Sequences were analyzed using PLACE which is an online analysis tool. (https://www.dna.affrc.go.jp/PLACE/?action=newplace) (Higo *et al*., 1999).

Results

Detection and confirmation of *OsRGLP1*: RNA extracted from the leaves of transgenic tomato was converted to cDNA. Primers specific to *OsRGLP1* was then used to determine the presence of the transgene. The expected band size was obtained as shown in (Fig. 1). **Fold change expression calculation using - ΔΔ Ct method:** The - ΔΔ Ct method was used to calculate the fold change expression of PR1, PR2 and PR5 between the transgenic and control tomato plants. As can be seen, the data suggests that there was a high expression of PR2 and PR5 protein genes in the transgenic plants. While there was little to no expression of PR1 protein gene (Fig. 2).

Bioinformatics analysis

Promoter analysis of pathogenesis related protein 1, 2, 5 and *OsRGLP1*: A total of about 148 Cis acting regulatory elements (CARES) were found in all the promoters analyzed and these were then classified based on their known biological functions, hormone response and tissue specificity as already established in literature (Figs. 3a, 3b, 3c and 3d).

Promoter analysis of pathogenesis related protein 1: Figure 4 shows the cis acting regulatory elements (CARES) and the position they occur in the promoter region of tomato PR1. As compared to other promoters under this study, relatively few CARES are found in tomato PR1. Major CARES found are highlighted.

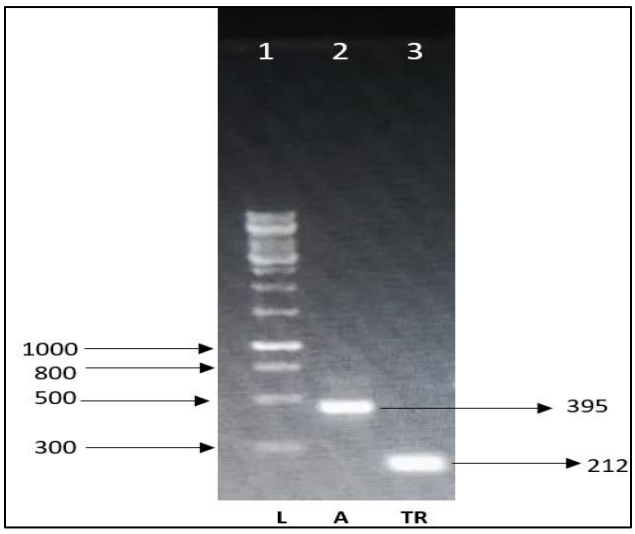

Fig. 1. Confirmation of the presence of *OsRGLP1*. 1.2% agarose gel stained with ethidium bromide showing the expression of *OsRGLP1* (Product size 212 bp) in Tomato. Lane 1: 1Kb Ladder (5 ul), Lane 2: cDNA with Actin primers (3 ul), Lane 3: cDNA with primers specific for *OsRGLP1* (3 ul).

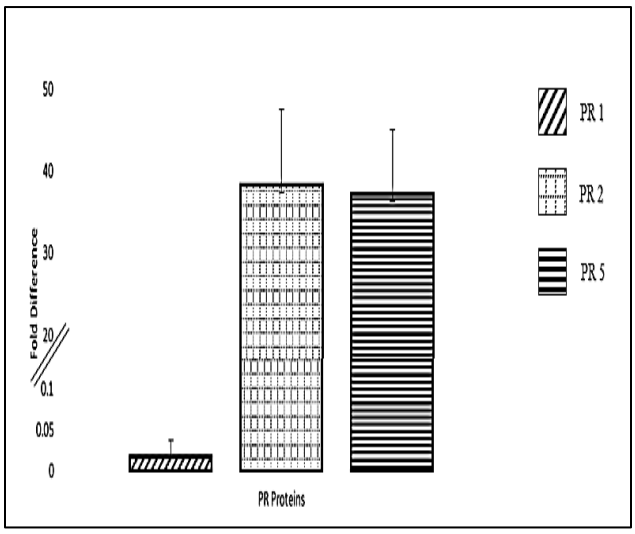

Fig. 2. Fold Change Expression of Tomato PR1, PR2 and PR5. qPCR output generated from triplicates of each PR gene was normalized against tomato actin as a reference gene. Fold change expression is presented here as Mean ± S.E.

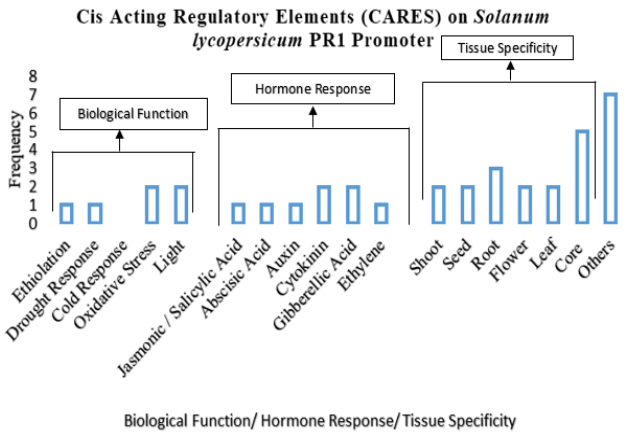

Fig. 3a. Cis Acting Regulatory Elements (CARES) found in the Promoter Region of PR1 classified on the basis of function.

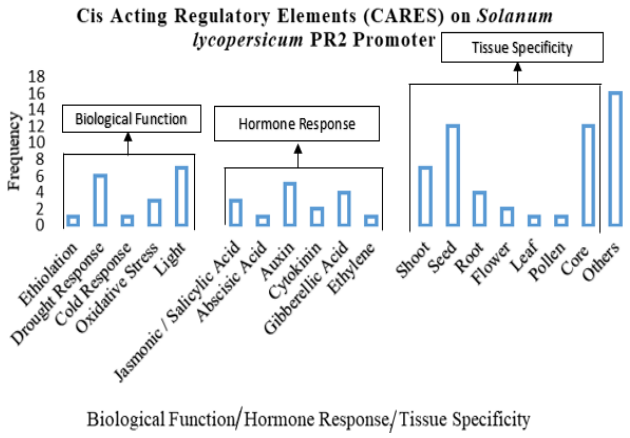

Fig. 3b. Cis Acting Regulatory Elements (CARES) found in the Promoter Region of PR2 classified on the basis of function.

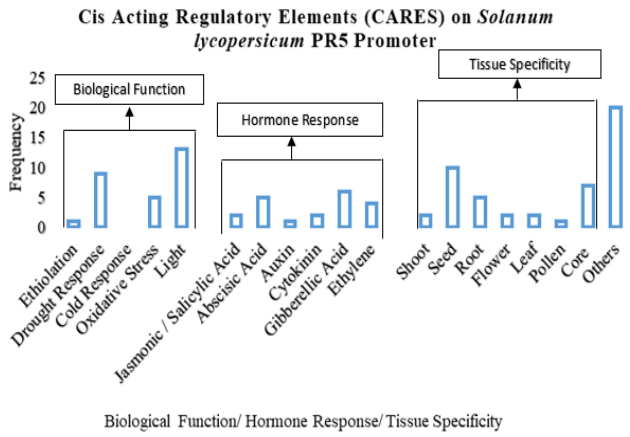

Fig. 3c. Cis Acting Regulatory Elements (CARES) found in the Promoter Region of PR5 classified on the basis of function.

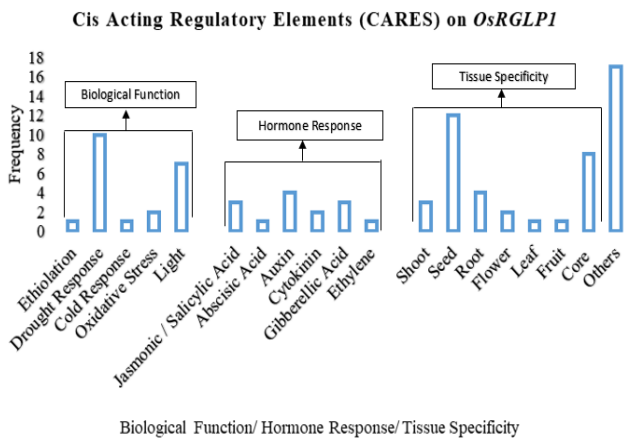

Fig. 3d. Cis Acting Regulatory Elements (CARES) found in the Promoter Region of *OsRGLP1* classified on the basis of function.

Promoter analysis of pathogenesis related protein 2: Figure 5 shows the cis acting regulatory elements (CARES) and the position they occur in the promoter region of tomato PR2. As shown in the figure, the region ranging from -600 to -1500 upstream of the transcription start site contains more elements as compared to others.

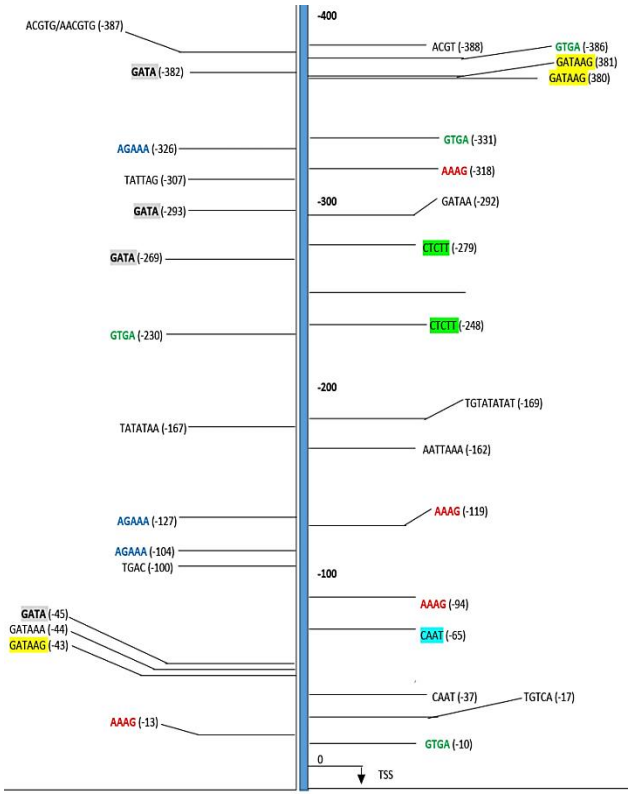

Fig. 4. Position of CARES found on the promoter region of tomato PR1 (391bp). For clarity sake, only CARES found on the minus strand are shown.

Promoter analysis of pathogenesis related protein 5: Figure 6 shows the cis acting regulatory elements (CARES) and the position they occur in the promoter region of tomato PR5. Just like PR2, the promoter region of tomato PR1 contains many CARES with majority of them occupying -500 to -1500 upstream of the TSS.

Promoter analysis of *OsRGLP 1*: Figure 7 shows the individual cis acting regulatory elements (CARES) found in the promoter region of *OsRGLP1*. In this case, a majority of the CARES on the minus strand of *OsRGLP1* promoter were seen from 0 to -1000 base pairs.

Summary of all CARES in the promoters analyzed: (Table 1) shows all the CARES found in the promoters analyzed. As shown, some elements such as DOFCOREZM, NODCON1GM and GTGANTG10 were found in all promoters analyzed. While others were unique to individual promoters.

Discussion

PR proteins induction in plants has been established to follow a SA dependent pathway or a JA dependent pathway (Schmiesing *et al*., 2016). To determine if the transgene *OsRGLP1* has an effect on the expression of PR proteins, we analyzed the PR transcript levels in transgenic tomato plants and compared them with those of wild type tomato plants using quantitative polymerase chain reaction (qPCR). There was a strong transcription upregulation of PR2 and PR5 in the transgenic plants as compared to the wild type control in our study. While in the case of PR1, there was no significant change in PR1 expression in both transgenic and control plants. Since it has been previously established that PR1, PR2 and PR5 are SA dependent genes (Knecht *et al*., 2010), therefore differences in expression of PR levels in the transgenic and wildtype control plants can be accounted for by the transgene (*OsRGLP1*). *OsRGLP1* have been thoroughly studied by different groups over the years (Yasmin *et al*., 2015; Majeed *et al*., 2018; Ilyas *et al*., 2019) and thus it has been established that the product of this gene is hydrogen peroxide (H_2O_2). Hydrogen peroxide have been shown to have different positive effects in plants. One of such includes the production of oxygen (O2) for metabolic activities (Katzman *et al*., 2001). Chien (1994) also illustrated their role in seed germination where they help in cracking hard seeds, thereby allowing them to interact with water. Besides all these positive effects of H_2O_2 in plants, of interest to us is their relationship with salicylic acid (SA) levels. Different research groups have since suggested that H_2O_2 increases SA levels, thereby acting as a second messenger for systemic acquired resistance (SAR) (Alvarez *et al*., 1998; Chamnongpol *et al*., 1998).

Putting all of these together, though the product of *OsRGLP1* is H_2O_2 which is known to increase SA levels, thereby inducing the synthesis of SA dependent PR genes (PR1, PR2 and PR5), why then was there no fold change difference in the expression of PR1 in the transgenic plant? A number of reasons may account for this. One of these includes the role of histone deacetylases. Since healthy transgenic tomato plants were used in this study, this means the plants were in a stress free and unchallenged conditions. It has been shown that HDA19 usually modifies the chromatin of PR1 genes into a repressive state, thereby ensuring a low expression of PR1 genes in an unchallenged condition (Choi *et al*., 2012). Also, different research groups have revealed that cis elements such as TGA 2 and TGA 5 can act as transcriptional repressors or activators of PR1 depending if the plant is in an infected or uninfected condition (Rochon *et al*., 2006; Kesarwani *et al*., 2007; Boyle *et al*., 2009). Suppressing the expression of PR1 could be important because it prevents the uncalled activation of defense responses thereby saving limited resources to be directed towards successful plant growth and development. To this end, we can suggest that the H_2O_2 product from *OsRGLP1* has no effect on PR1 gene expression in a tomato plant under unchallenged conditions. Similarly, the difference observed here in PR2 and PR5 expression in transgenic and wild type tomato plant is likely caused by the product of *OsRGLP1* which induced SA levels and thereby prompted their higher fold change expression.

We also tried to explore the cis acting regulatory elements (CARES) present in the promoter regions of PR class 1, 2, 5 and *OsRGLP1*. About 1.5kbp (PR2, PR5 and *OsRGLP1*) and 350bp (PR1) upstream of the promoter regions were analyzed using PLACE and a total of 148 cis acting regulatory elements (CARES) were identified. Of this, 25 of them were found to be common in all these promoters.

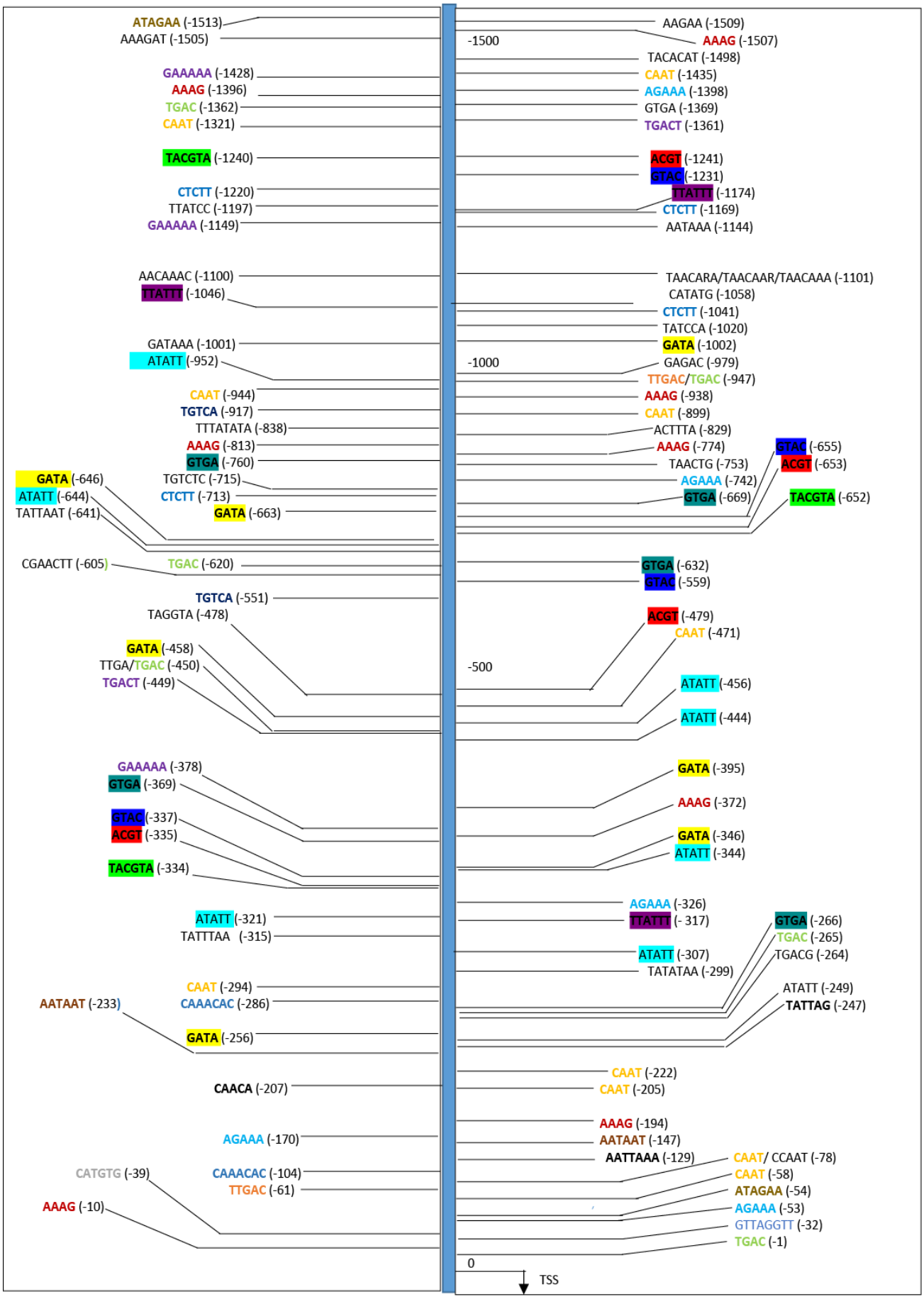

Fig. 5. Position of CARES found on the promoter region of tomato PR2 (1536bp). For clarity sake only CARES found on the minus strand are shown.

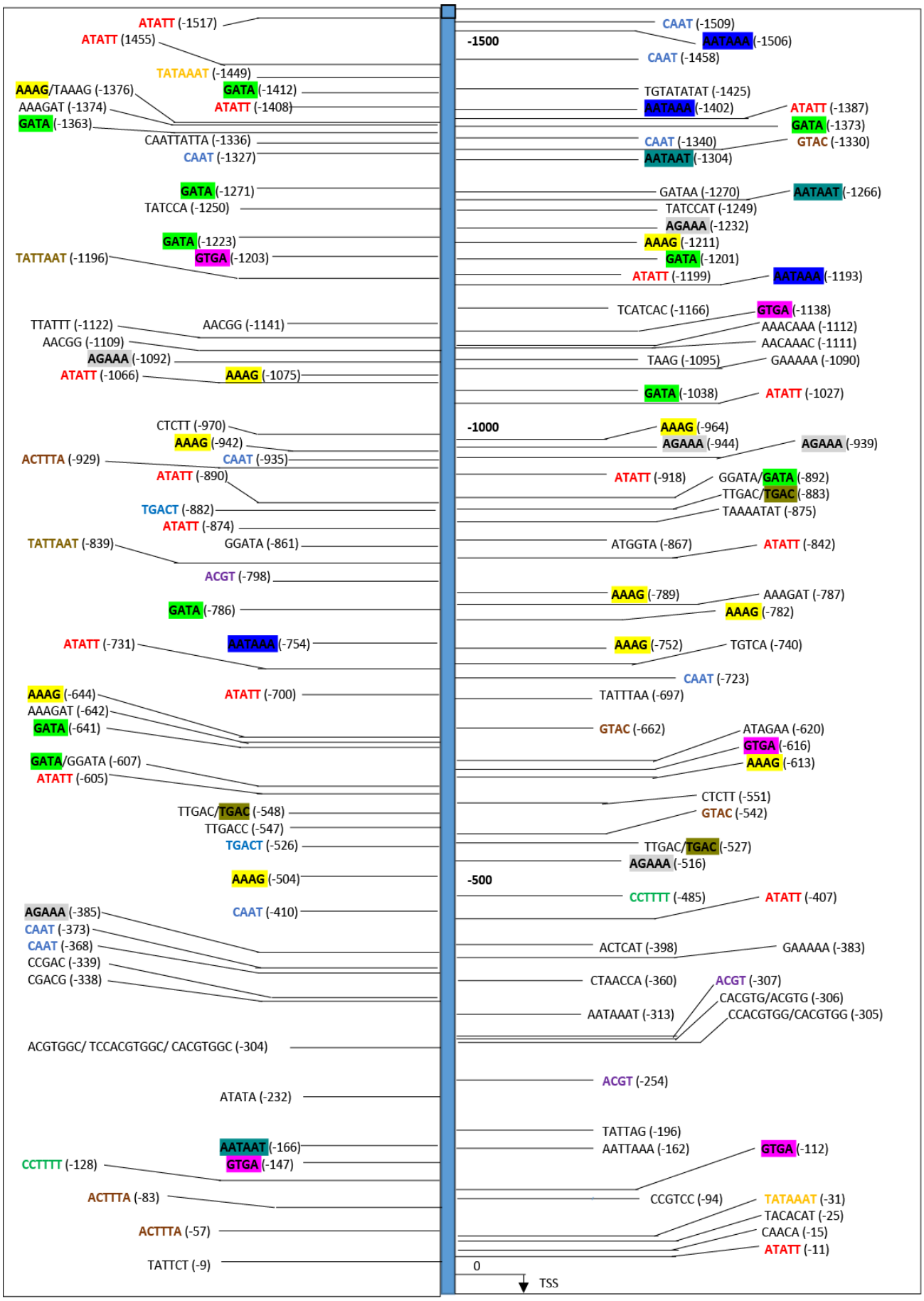

Fig. 6. Position of CARES found on the promoter region of tomato PR5 (1500bp). For clarity sake only CARES found on the minus strand are shown.

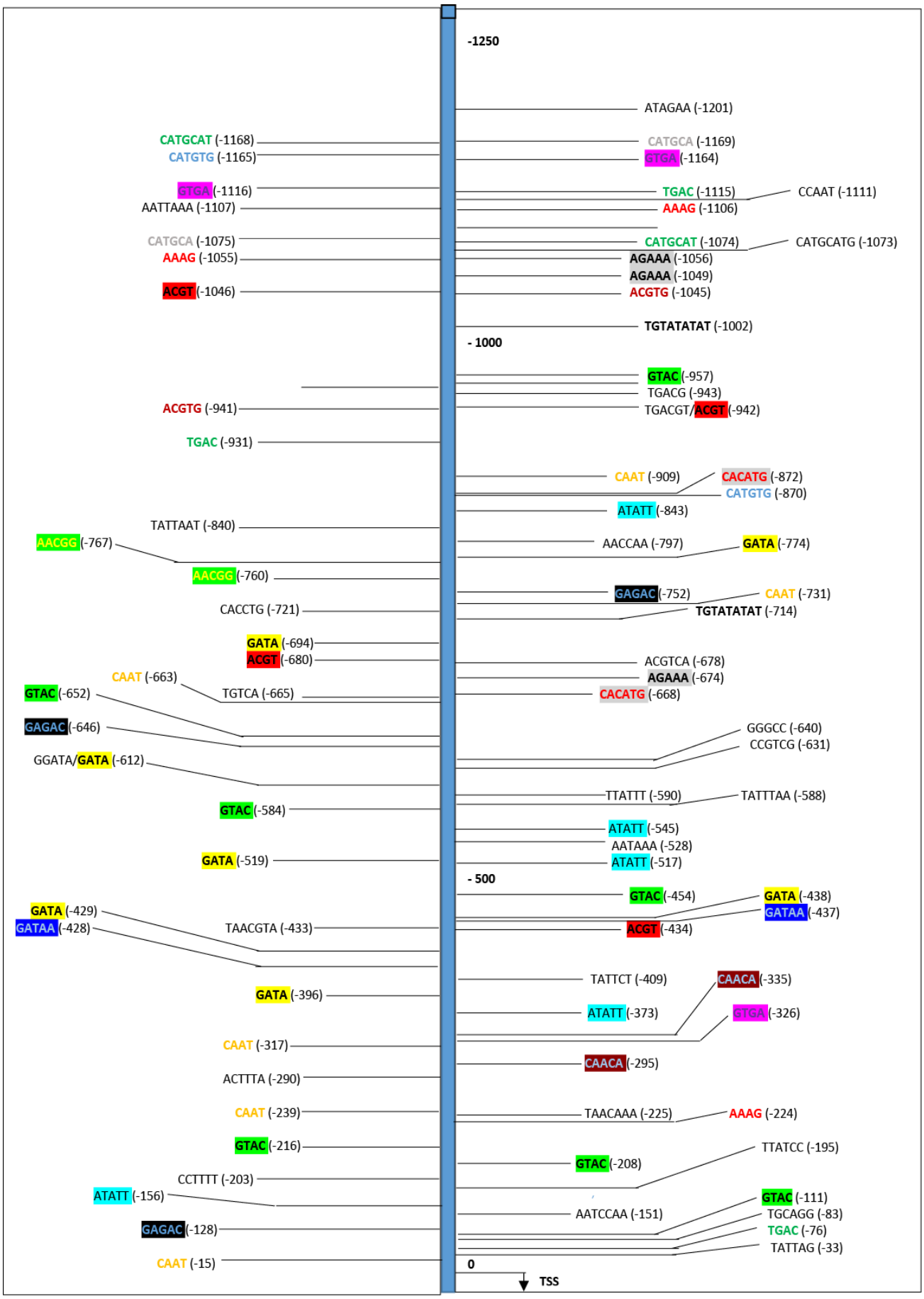

Fig. 7. Position of CARES found on the promoter region of *OsRGLP1* (1201bp). For clarity sake, only CARES found on the minus strand are shown.

Table 1. Summary providing comparison of all CARES in the promoters analyzed.

Elements found in all 3 promoters (PR 1, PR 2 and PR 5)	Elements found only in PR 1 promoter	Elements found only in PR 2 promoter	Elements found only in PR 5 promoter	Elements found in *OsRGLP1* promoter and all 3 promoters	Elements found only in *OsRGLP 1* promoter
DOFCOREZM	**IBOXCORENT**	**WBOXNTCHN48**	**-300ELEMENT**	**DOFCOREZM**	**SEBFCONSSTPR10A**
NODCON1GM	IBOX	MYB26PS	EMHVCHORD	NODCON1GM	TGTCACACMCUCUMISIN
OSE1ROOTNODULE	T/GBOXATPIN2	MYBPLANT	INRNTPSADB	OSE1ROOTNODULE	SURECOREATSULTR11
GTGANTG10		CANBNNAPA	PALBOXAPC	GTGANTG10	INTRONLOWER
ACGTATERD1		MYCATERD1	CARGATCONSENSUS	ACGTATERD1	ERELEE4
WBOXATNPR1		MYCATRD22	SEF1MOTIF	WBOXATNPR1	GARE2OSREP1
BIHD1OS		CCAATBOX1	GBOXLERBCS	BIHD1OS	HEXAMERATH4
WRKY71OS		2SSEEDPROTBANAPA	BOXIIPCCHS	WRKY71OS	RBCSCONSENSUS
CAATBOX1		ASF1MOTIFCAMV	LREBOXIIPCCHS1	CAATBOX1	TGACGTVMAMY
NODCON2GM		PRECONSCRHSP70A	ACGTABREMOTIFA2OSEM	NODCON2GM	RAV1BAT
ARR1AT		ACGTABOX	ABREATCONSENSUS	ARR1AT	NTBBF1ARROLB
OSE2ROOTNODULE		ARFAT	SORLIP1AT	OSE2ROOTNODULE	RYREPEATBNNAPA
IBOXCORE		MYB2AT	EMBP1TAEM	IBOXCORE	BOXIINTPATPB
GATABOX		MYB2CONSENSUSAT	ABREZMRAB28	GATABOX	TATABOX4
PYRIMIDINEBOXOSRAMY1A		MYBCORE	IRO2OS	PYRIMIDINEBOXOSRAMY1A	REALPHALGLHCB21
POLLEN1LELAT52		TATAPVTRNALEU	ABRERATCAL	POLLEN1LELAT52	RYREPEATGMGY2
GT1CONSENSUS		GARE1OSREP1	WBBOXPCWRKY1	GT1CONSENSUS	HEXMOTIFTAH3H4
POLASIG2		CBFHV	CACGTGMOTIF	POLASIG2	RYREPEATVFLEB4
CARGCW8GAT		ARE1	MYBCOREATCYCB1, PREATPRODH	RHERPATEXPA7	RYREPEATLEGUMINBOX
TATABOXOSPAL		TBOXATGAPB	ANAERO3CONSENSUS	TATABOXOSPAL	
TAAAGSTKST1		P1BS	SITEIIATCYTC	TAAAGSTKST1	
DPBFCOREDCDC3		CATATGGMSAUR	UP1ATMSD, GT1MOTIFPSRBCS	DPBFCOREDCDC3	
SEF4MOTIFGM7S		SREATMSD	SORLIP2AT, -300CORE	SEF4MOTIFGM7S	
CPBCSPOR		GT1CORE	AMYBOX2	CPBCSPOR	
POLASIG3		PYRIMIDINEBOXHVEPB1	ATHB6COREAT, CGACGOSAMY3	POLASIG3	
RHERPATEXPA7		AMMORESIVDCRNIA1	ELRECOREPCRP1		
		TATABOX2	AGCBOXNPGLB		
			GCCCORE		
			MARARS		
			LTRECOREATCOR15		
			ANAERO2CONSENSUS		

In PR1 promoter, the most abundant element found was the CACTFTPPCA1 which appears 11 times. This element was also the most abundant in a similar study where the promoter of a rice sperm cell was analyzed (Sharma *et al*., 2011). The CACTFTPPCA1 has a sequence of YACT where Y could be either of T/C is known to account for the mesophyll-specific expression of phosphoenolpyruvate carboxylase gene in some plants (C4). Other CARES such as DOFCOREZM, CAATBOX1, and GATABOX were associated with tissue specific responses such as shoot, seed and leaf respectively (Iqbal *et al*., 2017). This may basically buttress the fact that PR1 expression can be triggered at any developmental stage of the plant. Similarly, elements responsive to different hormones such as jasmonic acid, salicylic acid, gibberellic acid, cytokinin, ethylene and auxin were also present. Furthermore, elements such as IBOXCORENT, IBOX and T/GBOXATPIN2 were unique and seen only in the promoter of PR1.

For tomato PR2 promoter, a vast number of the CARES found have tissue specific activities and seed specific expression. This can easily be accounted for by the fact that PR2 proteins are glucanases and their activities will be highly required for the emergence of a seed radicle (Barba-Espin *et al*., 2010). In terms of hormone responsiveness, the most abundant CARES in this category were responsive to auxins and cytokines. Again, this is linked to PR2 and its role as glucanases because auxins and cytokines have been shown to regulate the processes of cell enlargement and cell division, both of which play important roles in the germination of seeds. (Barba-Espin *et al*., 2010). In addition to the above elements, other elements such as MYBPLANT, CCAATBOX1, CBFHV and ARE1 are unique and were only found in the promoter of tomato PR2.

PR5 promoter analysis shows CARES that play different roles in drought response, oxidative stress, hormonal response and tissue specificity. Light responsive elements such as INRNTPSADB, SORLIP1AT and SORLIP2AT were also found to be unique and occurred only in the promoter of tomato PR5. The INRNTPSADB motif with a sequence of YTCANTYY is known as an initiator and usually works in a TATA less promoter (Salehi *et al*., 2017). This motif is also found in the promoter of *Zmcyc5* which is a defence gene induced in *Zea mays* by biotic and abiotic stresses (Salehi *et al*., 2017).

The promoter of *OsRGLP1* was also analysed to find common elements among all promoters. It was observed that most CARES were related to drought response, light stress response and tissue specificity. CARES unique to *OsRGLP1* included, INTRONLOWER, RYREPEATLEGU MINBOX, RYREPEATGMGY2 and BOXIINTPATPB. It has previously been established that these motifs are associated with storage protein accumulation which are seed maturation processes (Zaidi *et al*., 2017) and well suited to the functions of GLPs. A class of bZIP transcription factors that binds to the "TGACG" element in the promoters are referred to as the TGA transcription family (Banerjee & Roychoudhury, 2017). This element was found 2, 13, 6 and 10 times in the promoter of tomato PR1, PR2, PR5 and *OsRGLP1* respectively. Contrary to Pape *et al*., (2010) where they stated that the promoter of PR2 and PR5 genes do not contain TGA-binding sites in Arabidopsis, the promoter of tomato PR2 and PR5 did contain TGA binding sites. The TGACG element are known to play roles in many transcriptional responses regulated by hormonal levels. Besides activating SA dependent gene expression, they have been shown to have both positive and negative effects on jasmonic/ethylene dependent responses (Kaur *et al*., 2017). In response to increases in SA levels upon pathogen infection, a ternary complex between TGA, NPR 1 and DNA is formed and this activates the transcription of PR1 (Caarls *et al*., 2015).

The AAAG motif is associated with the DOFCOREZM element and is usually known as the binding site of DOF proteins. DOF proteins are types of zinc finger regulatory proteins that plays important roles in plant gene expression (Konishi & Yanagisawa 2007). This element was found 9, 13, 25 and 12 times on the promoter regions of PR1, PR2, PR5 and *OsRGLP1* respectively. Such large number of element repeats in the promoters of PR protein could be due to the importance of DOF proteins which have been ubiquitously found in all plant organs studied so far (Cai *et al*., 2013). BIHD1OS element with sequence TGTCA which is common in all the promoters analysed was found 2 times on both *OsRGLP1* and the promoter of tomato PR1, 4 times on PR2 and only once on the promoter of PR5. This element is involved in plant disease resistance responses (Salehi *et al*., 2017) and is the binding site of the BELL homeodomain transcription factor which is usually associated with resistance response in rice (Sharma *et al*., 2011).

Conclusion

The expression of PR2 and PR5 was upregulated in transgenic tomato plants as compared to control. This clearly indicates that *OsRGLP1* could be used in conditions where a higher PR2 and PR5 expression is desirable. The promoter analysis of all three PR genes indicated that many conserved cis acting regulatory elements (CARES) were found in the promoters of all 3 PR genes analyzed. Furthermore, some CARES were common between all three PR genes and the promoter of *OsRGLP1*. This could possibly suggest that similar transcription factors are involved in the expression and regulation of all 4 genes. This information could be useful for understanding tomato plant promoter architecture as well as identifying the corresponding protein factors and transcription complexes required for their expression.

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References

Agarwal, P. and P.K. Agarwal. 2014. Pathogenesis related-10 proteins are small, structurally similar but with diverse role in stress signaling. *Mol. Boil. Rep.*, 41(2): 599-611.

Alvarez, M.E., R.I. Pennell, P.J. Meijer, A. Ishikawa, R.A. Dixon and C. Lamb. 1998. Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. *Cell*, 92(6): 773-784.

Banerjee, A. and A. Roychoudhury. 2017. Abscisic-acid-dependent basic leucine zipper (bZIP) transcription factors in plant abiotic stress. *Protoplasma*, 254(1): 3-16.

Barba-Espin, G., P. Diaz-Vivancos, M.J. Clemente-Moreno, A. Albacete, L. Faize, M. Faize, F. Pérez-Alfocea and J.A. Hernández. 2010. Interaction between hydrogen peroxide and plant hormones during germination and the early growth of pea seedlings. *Plant, Cell & Environ.,* 33(6): 981-94.

Boyle, P., E.L. Su, A. Rochon, H.L. Shearer, J. Murmu, J.Y. Chu, P.R. Fobert and C. Despres. 2009. The BTB/POZ domain of the Arabidopsis disease resistance protein NPR1 interacts with the repression domain of TGA2 to negate its function. *Plant Cell*, 21: 3700-3713.

Caarls, L., C.M. Pieterse and S. Van Wees. 2015. How salicylic acid takes transcriptional control over jasmonic acid signaling. *Front. Plant Sci.*, 6: 170.

Cai, X., Y. Zhang, C. Zhang, T. Zhang, T. Hu, J. Ye, J. Zhang, T. Wang, H. Li and Z. Ye. 2013. Genome-wide analysis of plant-specific Dof transcription factor family in tomato. *J. Integr. Plant Biol.,* 55(6): 552-566.

Chamnongpol, S., H. Willekens, W. Moeder, C. Langebartels, H. Sandermann, M. Van Montagu, D. Inzé and W. Van Camp. 1998. Defense activation and enhanced pathogen tolerance induced by H_2O_2 in transgenic tobacco. *Proc. Nat. Acad. Sci.*, 95(10): 5818-5823.

Chien, C.T. 1994. Mechanism of hydrogen peroxide in improving the germination of *Cinnamonum camphora* seed. *J. Exp. Bot.,* 44: 127-132.

Choi, S.M., H.R. Song, S.K. Han, M. Han, C.Y. Kim, J. Park, Y.H. Lee, J.S. Jeon, Y.S. Noh and B. Noh. 2012. HDA19 is required for the repression of salicylic acid biosynthesis and salicylic acid-mediated defense responses in Arabidopsis. *Plant J.*, 71(1): 135-146.

Goñi, O., M.T. Sanchez-Ballesta, C. Merodio and M.I. Escribano. 2010. Potent cryoprotective activity of cold and CO2-regulated cherimoya (*Annona cherimola*) endochitinase. *J. Plant Physiol.,* 167(14): 1119-1129.

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

Ilyas, M., W. Akhtar, S. Rehman, S.M.S. Naqvi and T. Mahmood. 2019. Functional characterization of the rice root Germin-like protein gene-1 (*OsRGLP1*) promoter in *Nicotiana tabacum*. 3. *Biotechnology*, 9(4): 130.

Iqbal, A., W. Tang, T. Wang and H. Wang. 2017. Identification and functional characterization of the promoter driving "xyloglucan endotransglucosylase/hydrolase gene (XET)" gene for root growth in the desert *Populus euphratica*. *S. Afr. J. Bot.*, 112: 437-446.

Katzman, L.S., A.G. Taylor and R.W. Langhans. 2001. Seed enhancements to improve spinach germination. *Hort. Sci.*, 36(5): 979-981.

Kaur, A., P.K. Pati, A.M. Pati and A.K. Nagpal. 2017. In-silico analysis of cis-acting regulatory elements of pathogenesis-related proteins of *Arabidopsis thaliana* and *Oryza sativa*. *Plo S One*, 12(9):e0184523.

Kesarwani, M., J. Yoo and X. Dong. 2007. Genetic interactions of TGA transcription factors in the regulation of pathogenesis-related genes and disease resistance in Arabidopsis. *Plant Physiol.,* 144(1): 336-346.

Kim, J.Y., J. Wu, S.J. Kwon, H. Oh, S.E. Lee, S.G. Kim, Y. Wang, G.K. Agrawal, R. Rakwal, K.Y. Kang and I.P. Ahn. 2014. Proteomics of rice and *Cochliobolus miyabeanus* fungal interaction: insight into proteins at intracellular and extracellular spaces. *Proteomics*, 14(20): 2307-2318.

Knecht, K., M. Seyffarth, C. Desel, T. Thurau, I. Sherameti, B. Lou, R. Oelmüller and D. Cai. 2010. Expression of BvGLP-1 encoding a germin-like protein from sugar beet in *Arabidopsis thaliana* leads to resistance against phytopathogenic fungi. *Mol. Plant-Microb. Inter.,* 23(4): 446-457.

Konishi, M. and S. Yanagisawa. 2007. Sequential activation of two Dof transcription factor gene promoters during vascular development in *Arabidopsis thaliana*. *Plant Physiol. Biochem.,* 45(8): 623-629.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 25(4): 402-408.

Majeed, N., B. Javaid, F. Deeba, S.M.S. Naqvi and D.S. Douches. 2018. Enhanced *Fusarium oxysporum* f. sp. tuberosi resistance in transgenic potato expressing a rice GLP superoxide dismutase gene. *Amer. J. Potato Res.,* 95(4): 383-394.

Pape, S., C. Thurow and C. Gatz. 2010. The Arabidopsis PR-1 promoter contains multiple integration sites for the coactivator NPR1 and the repressor SNI1. *Plant Physiol.*, 154(4): 1805-1818.

Ramos, M.V., R.S. de Oliveira, H.M. Pereira, F.B. Moreno, M.D. Lobo, L.M. Rebelo, J. Brandão-Neto, J.S. de Sousa, A.C. Monteiro-Moreira, C.D. Freitas and T.B. Grangeiro. 2015. Crystal structure of an antifungal osmotin-like protein from *Calotropis procera* and its effects on *Fusarium solani* spores, as revealed by atomic force microscopy: insights into the mechanism of action. *Phytochem.*, 119: 5-18.

Richards, E.J. 1997. Preparation of plant DNA using CTAB. - In: (Eds.): Ausubel, F., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, K. Struhl. *Short Protocols in Molecular Biology*. John Wiley, New York, pp. 2.10-2.11.

Rochon, A., P. Boyle, T. Wignes, P.R. Fobert and C. Després. 2006. The coactivator function of Arabidopsis NPR1 requires the core of its BTB/POZ domain and the oxidation of C-terminal cysteines. *The Plant Cell*, 18(12): 3670-3685.

Salehi, H., B. Bahramnejad and M. Majdi. 2017. Induction of two cyclotide-like genes Zmcyc1 and Zmcyc5 by abiotic and biotic stresses in *Zea mays*. *Acta. Physiol. Plantarum*, 39(6): 131.

Schmiesing, A., A. Emonet, C. Gouhier-Darimont and P. Reymond. 2016. Arabidopsis MYC transcription factors are the target of hormonal salicylic acid/jasmonic acid cross talk in response to *Pieris brassicae* egg extract. *Plant Physiol.*, 170(4): 2432-2443.

Sharma, N., S.D. Russell, P.L. Bhalla and M.B. Singh. 2011. Putative cis-regulatory elements in genes highly expressed in rice sperm cells. *BMC Res. Notes*, 4(1): 319.

Sudisha, J., R.G. Sharathchandra, K.N. Amruthesh, A. Kumar and H.S. Shetty. 2011. Pathogenesis related proteins in plant defense response. In *Plant defence: Biolog. Cont*. Springer, Dordrecht. pp. 379-403.

Ullah, A., A. Hussain, M. Shaban, A. H. Khan, M. Alariqi, S. Gul, Z. Jun, S. Lin, J. Li, S. Jin and M.F.H. Munis. 2018. Osmotin: A plant defense tool against biotic and abiotic stresses. *Plant Physiol. Biochem.,* 123: 149-159.

Yasmin, T., A. Mumtaz, T. Mahmood, M.Z. Hyder and S.M.S. Naqvi. 2015. A germin-like protein gene of rice increased superoxide dismutase activity in transformed tobacco. *Biol. Plantarum*, 59(3): 456-462.

Zaidi, M.A., S.J. O'Leary, S. Wu, D. Chabot, S. Gleddie, A. Laroche, F. Eudes and L.S. Robert. 2017. Investigating Triticeae anther gene promoter activity in transgenic *Brachypodium distachyon*. *Planta*, 245(2): 385-396.

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