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Full Length Research Paper

Comparative analysis of regulatory elements in different germin-like protein gene promoters

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Germin and germin-like proteins (GLPs) the members of cupin superfamily of proteins, which are functionally most diverse proteins. Germin and GLPs have some unique features as they are highly resistant to proteases and to degradation by heat, high pH and detergents like Sodium dodecyl Sulphate (SDS). They are water soluble extracellular enzymatic protein that may also have Oxalate Oxidase (OxO), Superoxide dismutase (SOD) or ADP-glucose pyrophosphate or phosphodiestrase (AGPPase) activities. At the moment seven GLP gene promoter from different organisms have been studied and published. These all promoter sequences have been analyzed in this study. It was observed that these promoters have important regulatory elements, which are involved in various important functions. These elements have been compared on the basis of location, copy number, and distributed on positive and negative strands. It was also observed that some of these elements are common and remained conserved among all GLP promoters during evolution. Such regulatory elements are commonly observed in seed storage proteins, dehydration in response to light, senescence observed on exposure to dark and in elements specific for expression in pollen. Moreover, these common elements are reported to be expressed under environmental stresses (salt and pathogen attack) and to growth regulators.

Key words: Germin-like protein gene, regulatory elements mapping, promoter.

INTRODUCTION

Germin and germin-like proteins (GLPs) is a member of plant protein family, highly resistant to proteases and inactivation by agents like heat, SDS and extreme pH. GLPs are ubiquitous plant proteins found in plant kingdom, that is, they have been reported from mono-cotyledonous and dicotyledonous Angiosperms, Gymno-sperms and also from bryophytes and algae (Dunwell, 1998, 2008). Despite considerable sequence variation (< 20% similarity), germins and GLPs share key amino acid residues thereby sharing a common structure (Dunwell, 1998, Dunwell et al., 2000, 2001, 2008). Despite func-tional diversity, their structure is found to be similar to the members of cupin superfamily like isomerases, cyclases, dioxygenases, sugar or auxin-binding proteins as well as monomeric or dimeric globulin seed storage proteins, such as phaseolin (Dunwell et al., 2000). Some GLPs have been reported to possess superoxide dismutase (SOD) or phosphodiesterase activity (Bernier and Berna, 2001) that is in addition to their obvious oxalate oxidase activity (Caliskan, 2000), which suggests the involvement of germins/GLPs in apoplastic detoxification. OxO acts on oxalic acid secreted by phytopathogens like ***Sclerotinia sclerotiorum*** whereas SOD acts as free radical scavenger and in either reaction H_2O_2 is produced. Moreover, in non-enzymatic biochemical activities, GLPs are found to be involved for example their role as auxin-binding proteins in peach or serine protease inhibitors in wheat (Segarra et al., 2003).

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Germins and GLPs are neither restricted to cereal only nor are expressed specifically during germination and are often expressed during the early growth stages in wheat embryos due to which it was termed “germin” (Thompson and Lane 1980). Germin and GLPs are expressed at different stages of development. A GLP gene from ***Zea mays*** (***Zm***GLP1) was reported from green tissue of the plant (Fan et al., 2005) whose transcript was observed in higher quantities in young leaf tissue than in mature leaves (Fan et al., 2005). Further, GLPs have also been reported from cassava roots during a study at proteomic levels (Sheffield et al., 2006).

It had already been established that germins and GLPs are expressed under stress whether biotic or abiotic. The role of germins and GLPs in plant defense had been studied in ***Capsicum annuum*** L. cv. Bugang that showed hypersensitive response (HR) when infected with Tobacco mosaic virus pathotype P0 (TMV-P_0) (Park et al***.,*** 2004). The performance of a native herbivore of ***Nicotiana attenuata*** was found to be enhanced by silencing the germin-like gene (Lou and Baldwin, 2006). The recovery of GLPs from ***Beta vulgaris*** seedlings germinated under stress support their probable role during stress (De Los Reyes and McGrath, 2003).

In present study, we have compared different GLP promoters on the basis of presence, number and location of regulatory elements. Earlier, two GLP promoter regions have been amplified, cloned, sequenced and analyzed and the findings have been reported as well (Mahmood et al., 2007; Yasmin et al., 2008).

MATERIAL AND METHOD

Isolation of GLP promoter regions

The sequences of seven different GLP gene promoters ***Hv*** GerB (Accession No.DQ324801.1), ***Hv*** GerF (Accession No. DQ324800.1), ***Zm*** GLP1 (Accession No.AY394010.1), ***Pc*** GER1 (Accession No.AY077704.1), ***Ta*** GLP3 (Accession No. AY864922.1) and ***Os*** RGLP1 EU742684 were taken from Genbank. The size of all the promoters was purposely reduced to 1000 bp for uniformity.

Multiple Alignment of the Sequence

All the sequences were analyzed for checking the homo-logy among the promoter and phylogenetic tree was constructed by using online available program Clustal W (http://align.genome.jp).

Regulatory Element Analysis of GLP Promoters

The analysis of regulatory elements of various GLP promoters was done using web based program PLACE/Signal Scan (www.dna.affrc.go.jp) (Higo et al., 1999) and their functional importance was investigated from previously reported literature. Identified regulatory elements were compared with each other on the basis of presence or absence of a specific regulatory element, their copy number and position. On the basis of information gathered different maps were constructed for analyzing the position, number and location (negative or positive strand).

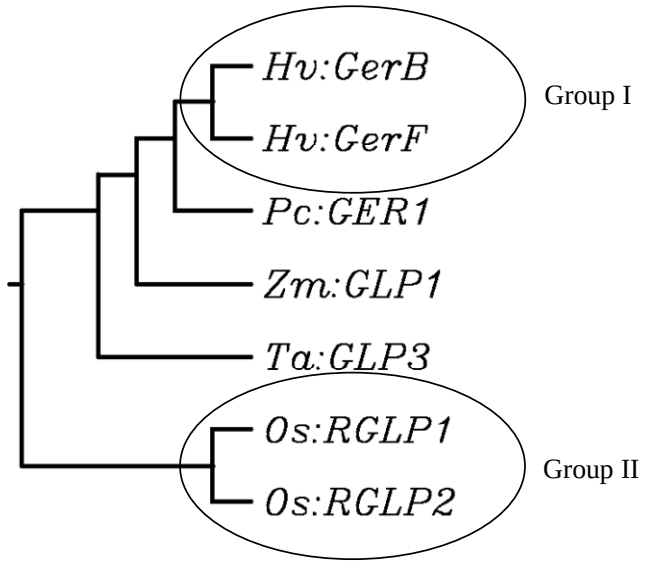

Figure 1. NJ tree showing similarities among seven GLP gene promoters.

RESULTS AND DISCUSSION

Isolation of GLP promoters

All GLP promoters from published data were picked up including two promoters from rice reported by our group (Mahmood et al., 2007, Yasmin et al., 2008). In total, seven different GLP promoter sequences were managed from data bases and for comparative analysis of these promoter sequences all the sequences were reduced to a constant length that is, 1000 bp.

Alignment of different GLP promoters

Clustal W is a general tool used for multiple alignments of DNA sequences. Seven different GLP promoter sequences were analyzed by Clustal W (http://align.genome.jp) and Neighbor Joining (NJ) tree was constructed (Figure 1). It was observed that Group I had 5 promoters ***Hv*** Ger B, ***Hv*** GerF, ***Pc***, GER1, ***Zm*** GLP1 and ***Ta*** GLP3 in NJ tree. In this group only ***Hv*** Ger B, ***Hv*** GerF have shown similar kind of closeness as Group II promoters ***Os*** RGLP1 and ***Os*** RGLP2 (Figure 1), however, their homo-logy was 69% while in ***Os*** RGLP1 and ***Os*** RGLP2 promoters, it was 21%. The remaining all promoters were observed to have close links with group I.

Identification/screening of regulatory elements

Different regulatory elements screened out with the help of “PLACE/Signal Scan”, an on online available tool (Higo et al., 1999) (www.dna.affrc.go.jp). All the sequences were found to contain a large number of ***cis***-acting

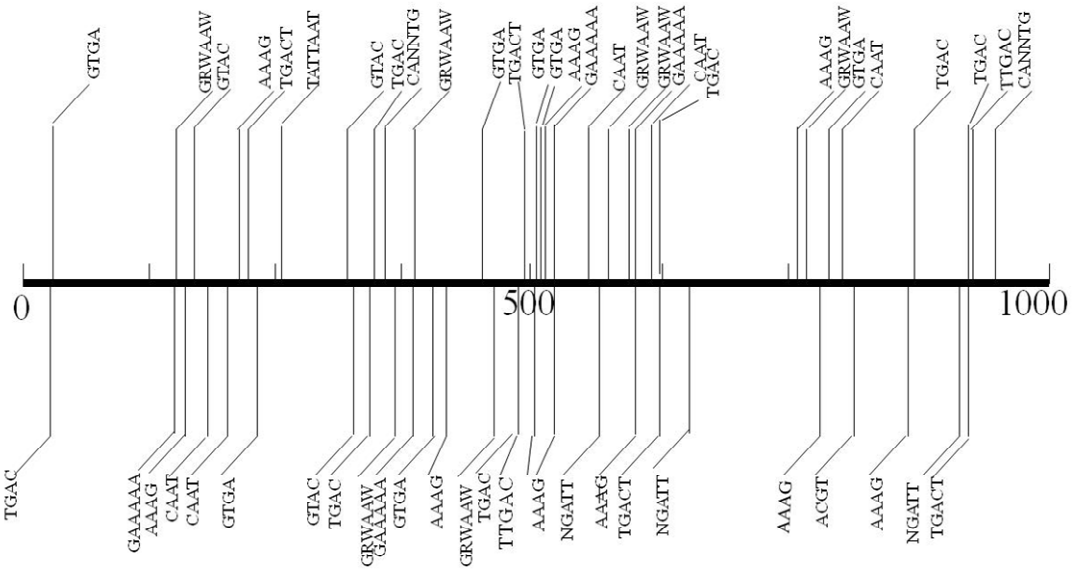

Figure 2a. Position of different common regulatory elements on positive strand of ***Os***RGLP2 promoter region.

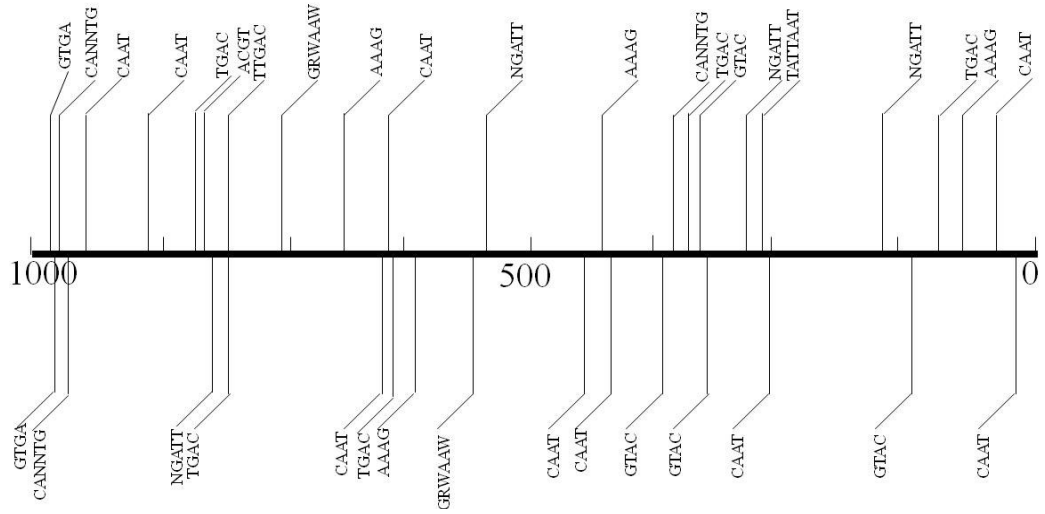

Figure 2b. Position of different common regulatory elements on negative strand of ***Os***RGLP2 promoter region.

regulatory elements, including the elements responsible for etiolation-induced expression, light-regulated gene expression, in pathogen and salt-induced gene expression, pollen specific expression and repressor of the gibberellin signaling pathway.

Mapping of regulatory element

Many potential regulatory elements were located using web based program, PLACE/Signal Scan having important functions. There were only 12 common regulatory elements among different GLP promoters. The regulatory elements located on positive strand and negative starnd were mapped separately (Figures 2a, 2b, Figures 3a, 3b, Figures 4a, 4b, Figures 5a, 5b, Figures 6a, 6b, Figures 7a, 7b, Figures 8a, 8b). It was also found that positive strand had more number of elements than negative except ***Pc*** GER1 and can be depicted from Figures 7a and 7b.

Functions of common promoter elements

The proposed function of the common regulatory elements were searched with the help of PLACE/Signal

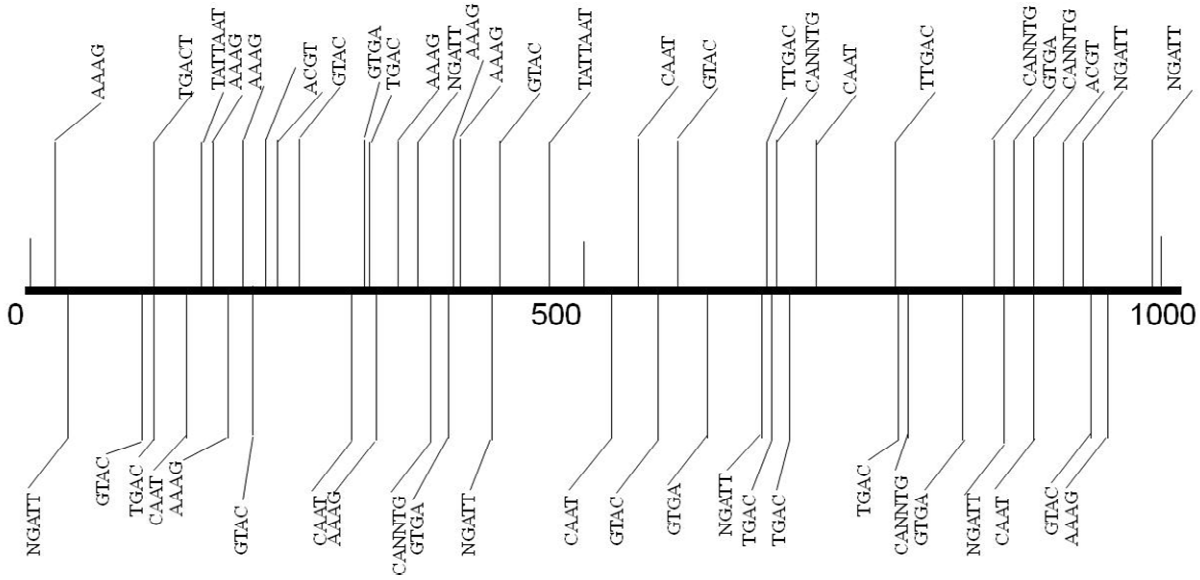

Figure 3a. Location of different common regulatory elements on positive strand of ***Os***RGLP1 promoter region.

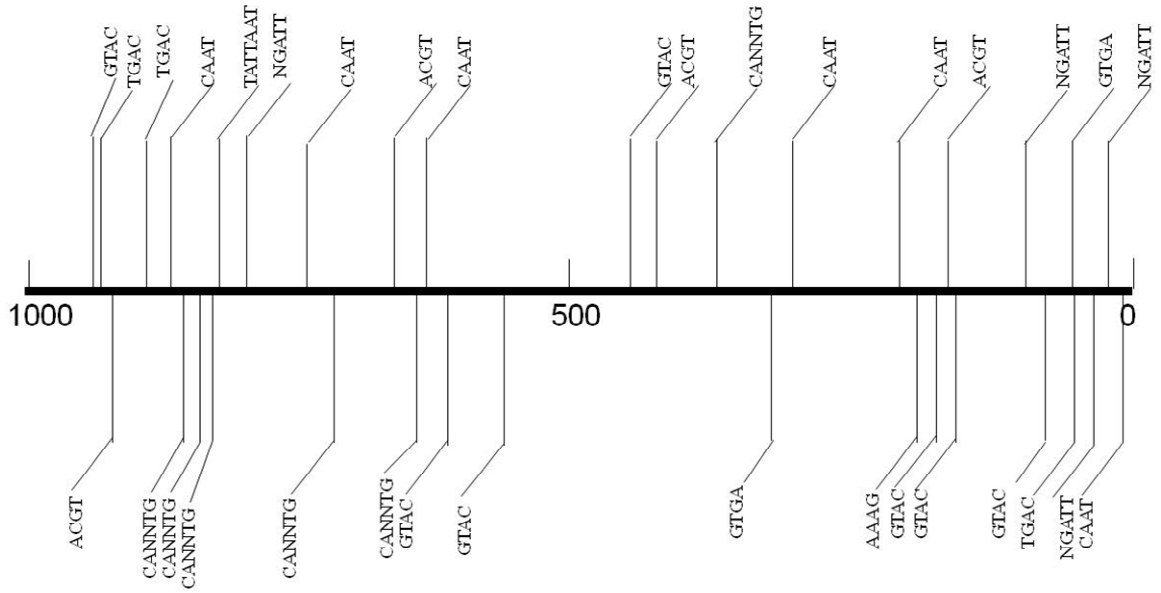

Figure 3b. Location of different common regulatory elements on negative strand of ***Os***RGLP1 promoter region.

Scan program and was observed to be involved in the expression of various important genes. The details of the common important factors are as follows.

DNA binding with one finger transcriptional factors (Dof): AAAG is particularly required for binding of Dof proteins and in this comparative study ***Ta*** GLP3 has highest copy number (18) and had random distribution on positive negative strands (Figures 8a and 8b). The role of Dof factors had been reported during expression of genes responsible for photosynthesis, seed storage and also for the gene stimulated by plant hormones and stress signals (Yanagisawa 1999; Zhang et al., 1995; De Paolis et al. 1996; Vicente-Carbajosa et al., 1997; Kisu et al.**,** 1998; Mena et al., 1998; Yanagisawa and Sheen 1998; Baumann et al., 1999).

ARR1

ARR1 belongs to type-B Arabidopsis response regulators (ARRs) and are responsible for early responses to cytokinins, which made possible by the combined efforts

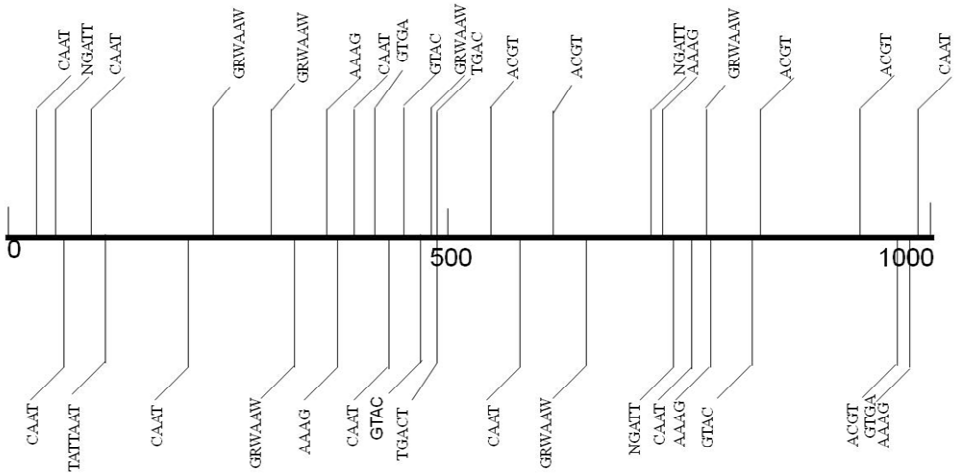

Figure 4a. Position of different common regulatory elements on positive strand of ***Hv*** GerB promoter region.

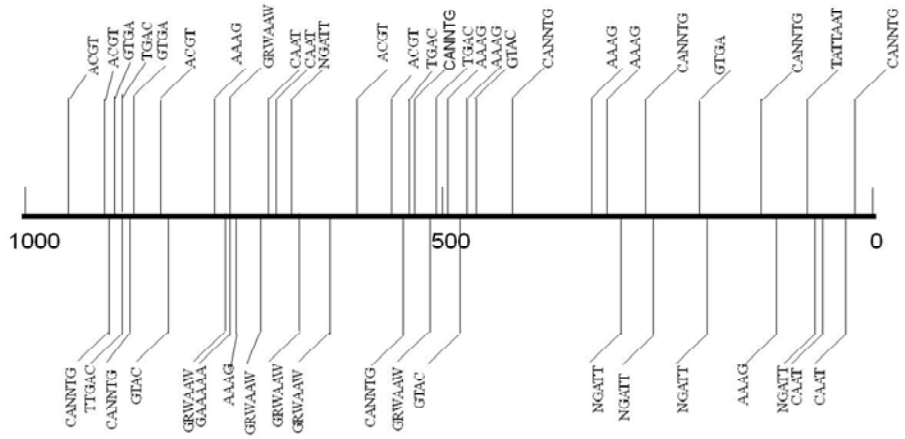

Figure 4b. Position of different common regulatory elements on negative strand of ***Hv*** GerB promoter region.

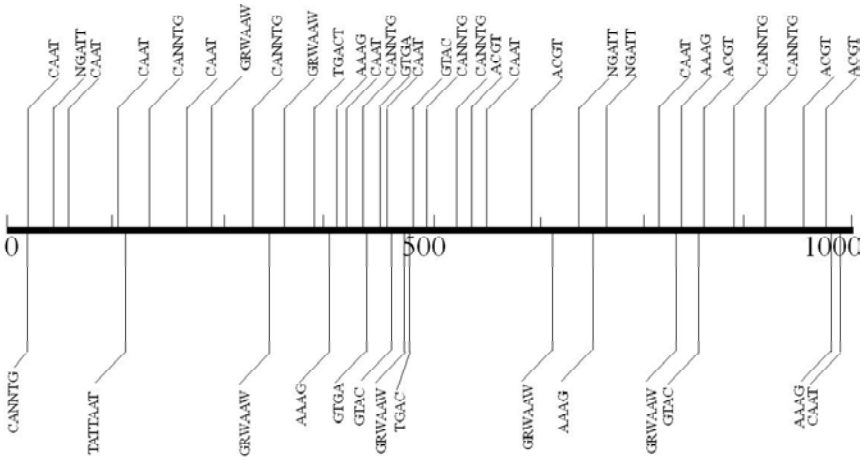

Figure 5a. Position of different common regulatory elements on positive strand of ***Hv*** GerF promoter region.

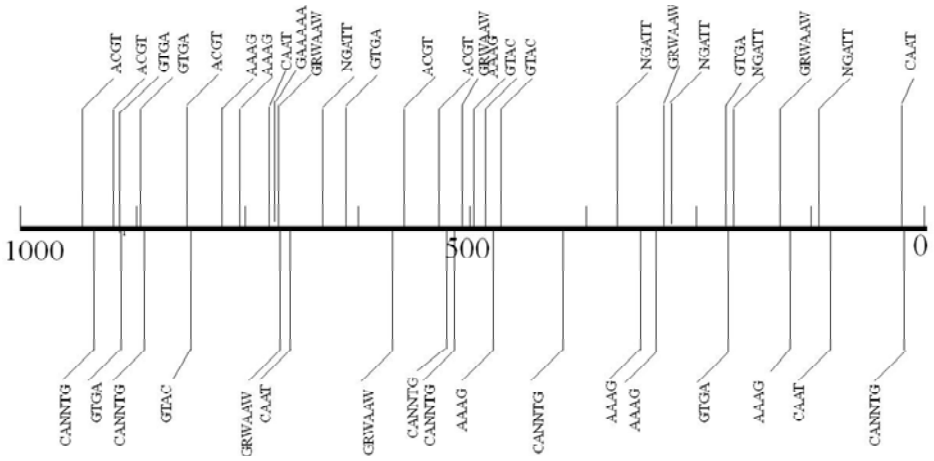

Figure 5b. Position of different common regulatory elements on negative strand of ***Hv*** GerF promoter region.

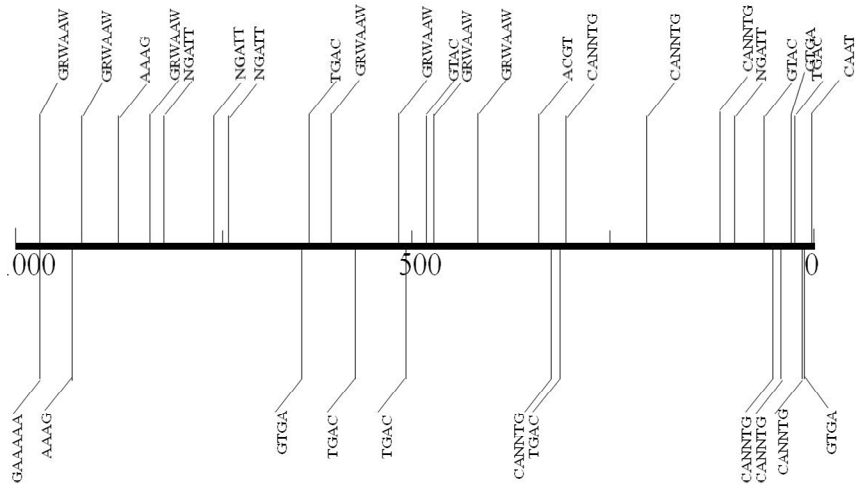

Figure 6a. Position of different common regulatory elements on positive strand of ***Zm*** GLP promoter region.

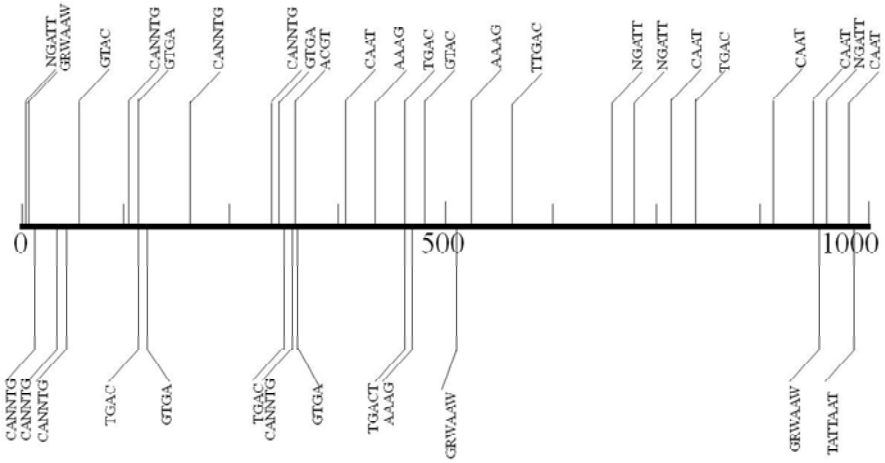

Figure 6b. Position of different common regulatory elements on negative strand of ***Zm*** GLP promoter region.

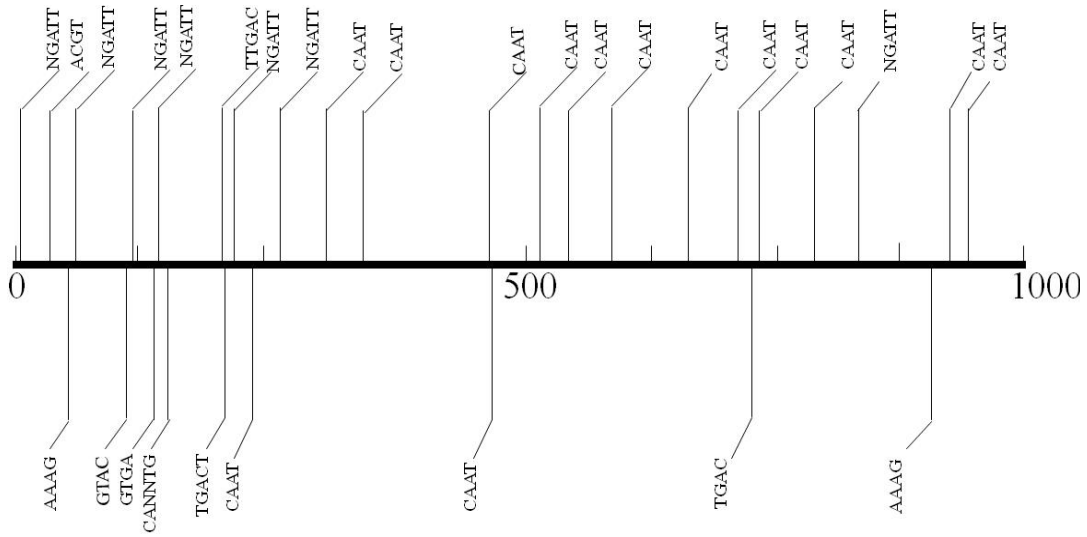

Figure 7a. Position of different common regulatory elements on positive strand of ***Pc*** GER1 promoter region.

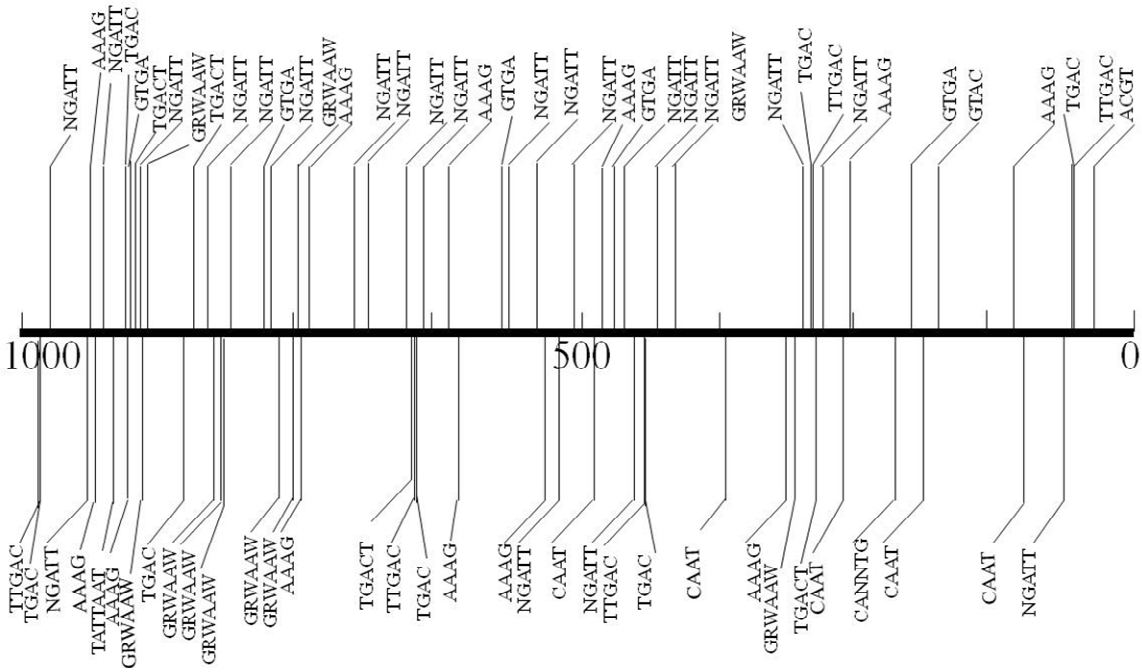

Figure 7b. Position of different common regulatory elements on negative strand of ***Pc*** GER1 promoter region.

of type-B ARRs and Myb–DNA binding domain as transcription activator (Imamura et al., 1999; Sakai et al., 2001; Haberer and Kieber 2002). ARR1 has also been proposed as a binding element of rice non-symbiotic haemoglobin-2 (NSHB) gene promoter region. ***Pc*** GER1 gene promoter has 29 copies of NGATT and more in number on positive strand (Figures 7b). It indicates its probable involvement for controlling expression of all above gene promoters in response to cytokinins.

W Box

The binding site for transcription factors that belong to WRKY family is W box [(T) TGAC(C/T)]. The W boxes form a class of ***cis*** -acting elements observed to be involved in genes expression as a result of pathogen attack for many plants (Wang et al., 1998). Further their role in development of trichomes and senescence is also noteworthy (Eulgem et al., 2000). W box was also

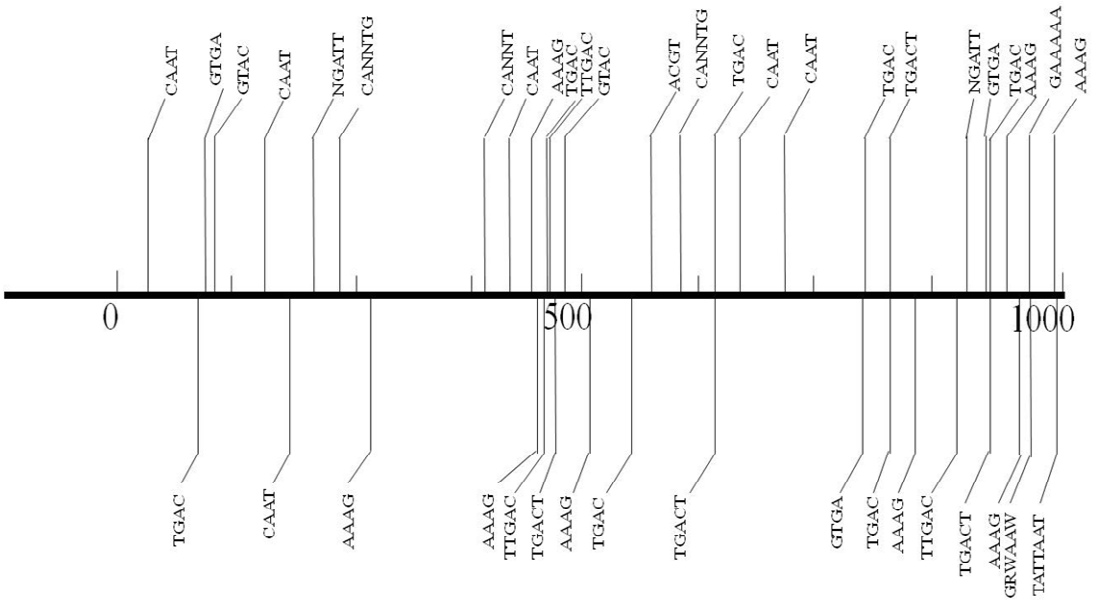

Figure 8a. Position of different common regulatory elements on positive strand of ***Ta*** GLP3promoter region.

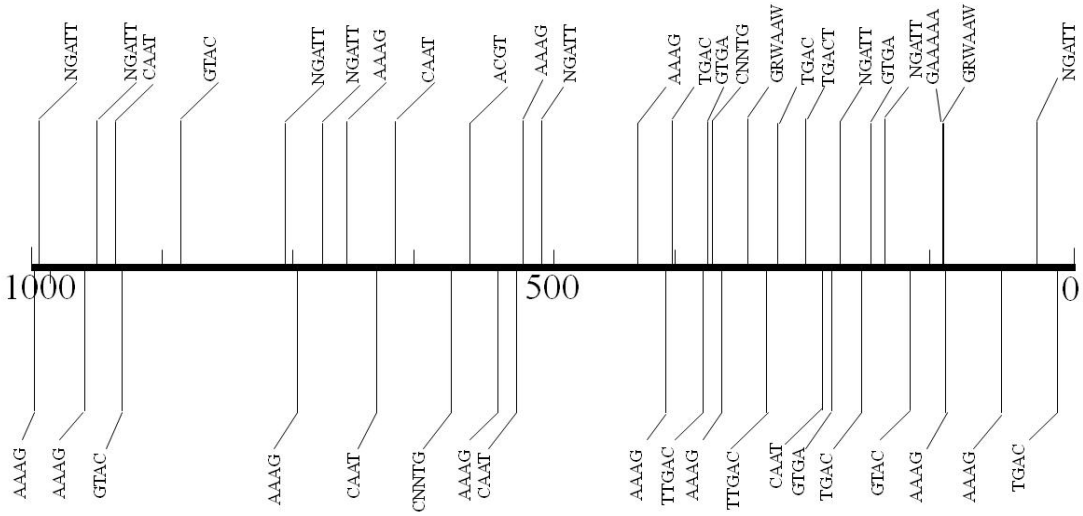

Figure 8b. Position of different common regulatory elements on negative strand of ***Ta*** GLP3 promoter region.

reported as enhancer for initiation of salicylic acid when tissue is injured and bacterial infection in ***Brassica oleracea***. ***Os***RGLP2 gene promoter has 14 copies of TGAC and heights in all (Figures 2a and 2b).

The TGAC element involved in gibberellin signaling pathway, was first reported from ***Oryza sativa*** (Eulgem et al., 1999). The specific binding of WRKY proteins from parsley was observed at TGAC-containing W box elements within the Pathogenesis-Related genes (Zhang et al., 2004). The TTGAC/TTGACC element, component of W box is found to be involved in pathogen related gene expression in Parsley (Rushton et al., 1996). This element has been reported in eleven copies from gene promoter ***Ta*** GLP3, which is the highest in this particular comparative study (Figures 8a and 8b).

GT elements

GT elements are involved in cell type-specific transcriptional regulation. Initial reports favor the presence of GT-1 ***cis***-elements in pea. It was further studies that when stresses like pathogen or salt is applied to the plant tissue, the genes with upstream GT element are

expressed robustly (Snedden and Fromm 1998, 2001). The obvious role of these elements in activation or repression of different photosynthesis related genes under light and dark conditions is notable. In this comparative study there are three sites having GT elements GT1CONSENSUS, GT1GMSCAM4 and GTGANTG10. GRWAAW is Consensus GT-1 binding site in many light-regulated genes (Terzaghi and Cashmore 1995). GAAAAA having "GT-1 motif" was reported from soybean (Glycine max) CaM isoform, SCaM-4 that is found to be involved in pathogen- and salt-induced SCaM-4 gene expression. ***Hv*** GerB has 13 numbers of copies. Further, “GTGA motif" from promoter region of tobacco late pollen gene g10 has also been reported.

ACGT Sequence

The role of ACGT under water stress and senescence has been repeatedly described and its contribution in the expression of erd1 (early responsive to dehydration ***Arabidopsis***) during etiolation is clear (Simpson et al., 2003). It was observed that ***Hv*** GerF has 10 copies of AGCT.

Conclusion

This comparative study of different GLP genes promoter sequences revealed that all seven GLP promoters have a wide range of ***cis***-acting elements, while some of them are common. Moreover, it was observed that these elements have diversified functions and have different number of copies at different locations (negative and positive strand).

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