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ARTICLE

In silico study of *cis*-acting elements revealing the plastid gene involved in oxidative phosphorylation are responsive to abiotic stresses

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ABSTRACT In order to study plastid gene response to abiotic stresses, the chloroplast genome of *Brassica nigra* and studied *cis*-acting elements were downloaded. All upstream regions of genes were determined and searched for the presence of known *cis*-acting elements. In these regions, 83 types of *cis*-acting elements were recognized. Unnamed elements (139 times), CAAT-box (96 times), and TATA-box (92 times) were in high frequency, whereas ATCC-motif, Box III, CE1, CE3, C-repeat/DRE, E2Fb, Gap-box, L-box, RY-element, and TGA-box occurred only one time. All of the *cis*-acting elements were grouped into seven categories, which 17% of *cis*-acting elements placed into abiotic and biotic-related elements. ARE (31 times) and LTR (21 times) elements were in high frequency. Among 42 genes with abiotic stress-related elements, 29 genes showed co-expression. Our results show that in response to anaerobic conditions and cold stress, chloroplast alters the genes-encoding proteins involved in complex I and V in oxidative phosphorylation pathway. This process, probably, is to reduce electron flow and convert NADPH and FADH forms to ATP form. These actions could decrease generating reactive oxygen species under stressful conditions. These findings could offer new insights on the strategies which chloroplasts take into account for preventing oxidative damage.
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Introduction

During evolution periods, plants have adapted molecular and cellular processes, which have enabled them to survive in constantly changing environments. Transcriptome analysis of plants have indicated that the expression of numerous genes is regulated by abiotic and biotic environmental stresses (Bray et al. 2000; Shinozaki et al. 2003). In such conditions, gene regulatory network is responsible to determine which sets of genes must be expressed, upregulated, downregulated or halted. Gene regulatory networks refer to a collection of molecules, which regulates a set of gene expression in a specific growth stage or in response to external stimuli. One of the important stages of gene expression regulation is at transcription level in which *cis*-acting elements and transcription factors (TF) mediate it. *Cis*-acting elements are strength (sequences) of DNA at the promoter region of a gene, which interact with transcription factors. TFs bound to *cis*-acting elements form the transcriptional initiation complex that activates RNA polymerase to start transcription process of specific genes. In this process, TFs act as molecular switchers to start transcription of the extraordinary gene. TFs themselves are activated in response to external stimuli such as salinity, drought, and temperature alterations and internal stimuli such as hormones (Yamaguchi-Shinozaki and Shinozaki 2005).

Many studies have focused on gene expression and regulatory networks of nucleus genes in response to various types of stimuli (Valliyodan and Nguyen 2006; Santos-Mendoza et al. 2008; Krasensky and Jonak 2012; Yoshida et al. 2014). The organelle genome, however, have been under less attention in the term of regulatory networks. Plastids are specific organelles responsible for photosynthesis and some important metabolic processes. They possess their own genetic material - found in plants and algal cells. It is believed that the origin of plastids in plant came back to an endosymbiotic relationship between plants and cyanobacteria a long time ago (McFadden and van Dooren 2004). The genome of chloroplast in higher plants are transcribed by two types of RNA polymerase, the nuclear-encoded RNA polymerase (NEP) and the plastid-encoded RNA polymerase (PEP). In *Arabidopsis*,

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NEP is phage-type RNA polymerase encoded in three forms, which two forms of them are targeted to chloroplast (Hess and Börner 1999; Maréchal et al. 2009). PEP encoded by the genes *rpoA, rpoB, rpoC1,* and *rpoC2* that are located on the plastid genome. This type of RNA polymerase in higher plants has some features of bacterial RNA polymerase (Liere et al. 2011). Like its counterpart in bacteria, the PEP of *Arabidopsis* is regulated by sigma-like transcription factors (*i.e.* SIG1 to SIG6) that are encoded by the nuclear genome (Yu et al. 2014). The studies on different organisms such as *Nicotiana tabacum* (Suzuki et al. 2004), spinach (Melonek et al. 2012), mustard (Steiner et al. 2011), and *Arabidopsis* (Pfalz and Pfannschmidt 2013) have indicated association of other proteins with PEP core subunits. Proteomic and biochemical analysis have identified some relationships and interactions between some of these nucleus-encoded proteins with regulatory events in chloroplast. Functionally, these proteins could be categorized into DNA/RNA binding proteins, thioredoxin proteins, kinases, ribosome proteins and proteins with unknown function (Steiner et al. 2011). All these proteins make a complex regulatory network that is regulated in response to environmental stimuli and chloroplast gene expression.

It has been shown that the transcriptional activity of plastid genes is affected by exogenous and endogenous factors such as light, temperature, hormones, plastid type, and developmental stage of the plant (Kim and Mullet 1995; Liere et al. 2011). The interaction of regulatory factors with core subunits of RNA polymerases and/or *cis*-regulatory elements is obviously necessary to change the transcription activities. Many of studies have been conducted to identify and role determination of *cis*-acting elements in regulatory networks in plastids using experimental studies. One of the well-known example is the *rbcl* gene in maize, which encodes the large subunit of ribulose-1,5-bisphosphate carboxylase oxygenase. The upstream region of this gene acts as binding site for the chloroplast DNA-binding factor 1 (Lam et al. 1988). Another example for *cis*-acting element is -3 to -32 promoter region of the *rbcl* gene in tobacco. The light-induced DNA-binding protein binds specifically to this segment in response to light to initiate the transcription of *rbcl* in light-dependent manner (Kim et al. 2002). The involvement of such conserved motifs have also been characterized in response to other factors such as blue light-responsive promoter of *psbD* gene (Sexton et al. 1990), temperature stress (Sun and Guo 2016) and water deficit (Stockinger et al. 1997).

In silico study of *cis*-acting elements have widely carried out for nucleus genes such as sucrose transporter gene families in rice (Ibraheem et al. 2010), polyphenol oxidase gene (Mahmood et al. 2015), and ascorbate glutathione pathway genes (Pandey et al. 2015). However, it seems that there is no report for studying *cis*-acting regulatory element of chloroplast genome using *in silico* approaches.

In this study, we used simple *in silico* approaches to analyze putative *cis*-acting element of promoter regions in the chloroplast genome of *Brassica nigra,* which was recently sequenced using the latest sequencing and assembling technologies (Seol et al. 2015). *Brassica nigra* belongs to the genus *Brassica* from the family Brassicaceae. The species of the genus *Brassica* are widely cultivated as food supplement of people, especially to produce vegetable oil all around the world (Bandehagh et al. 2011; Gharelo Shokri et al. 2016). All known *cis*-regulatory elements in plants have been searched in the chloroplast complete genome. The occurrence, functional categorization, distribution and frequency of *cis*-regulatory elements were determined. This study could indicate, whether the results of studying through this way agree with previous findings. If the answer is yes, as will show in this study, it could answer the question that; which plastid genes might undergo differential expression in response to other less known crucial factors such as abiotic stresses.

Materials and Methods

The whole genome of *Brassica nigra* chloroplast (NC_030450.1) was retrieved in FASTA format from National Center for Biotechnology Information (NCBI). Seol et al. (2015) generated this chloroplast genome sequence by *de novo* assembly using whole genome next generation sequences. The sequence consists of 153 633 bp and 114 genes including 80 protein-coding genes, 30 tRNA genes, and 4 rRNA genes.

Using the gene features recorded on NCBI, each gene sequence was separately extracted and saved in FASTA format. These sequences served as inputs on PlantPAN 2.0 (Chow et al. 2016), Promoter Prediction by Neural Network (Reese 2001), and PePPER (de Jong et al. 2012) to predict promoter regions and transcription starting sites (TSS) (Reese 2001). We searched possible promoter regions using the features of prokaryotes and eukaryotes, since chloroplast genome shows both features of prokaryote and eukaryote. A segment from 500 bp upstream and 45 bp downstream away from TSS was selected to detect known *cis*-regulatory elements (CRE) using PlantCARE, a database of plant promoters and their *cis*-acting regulatory elements (Lescot et al. 2002).

STRING 10.0 (http://string-db.org/) is an open source online bioinformatics tool that is used for studying co-expression of genes and gene ontology (GO) enrichment. The data setting was set as follows; minimum required interaction score: highest confidence (0.900), organism: *Arabidopsis thaliana* and disconnected node were hidden from the network. The results of GO enrichment illustrated as chart.

Results

Prediction of promoter regions and searching the cis-acting elements

The whole genome of the chloroplast (153 633 bp) was searched for occurrence of prokaryotic and eukaryotic promoters. Totally, 110 promoter regions including 50 eukaryotic and 60 prokaryotic promoters were identified. The average length of the promoter regions was 350 bp, although we selected 500 bp upstream and 20 bp downstream from TSS point. This was because of short length of upstream regions (<500 bp) for some predicted promoters. These regions were searched for presence of known *cis*-acting elements. The results indicated total 1125 known *cis*-acting elements in 83 types. Unnamed, CAAT-box and TATA-box sequences had the highest frequency with 139, 96 and 92 abundances, respectively. The *cis*-acting elements ATCC-motif, Box III, CE1, CE3, C-repeat/DRE, E2Fb, Gap-box, L-box, RY-element, and TGA-box occurred only one time.

The functional categorization of the cis-acting elements

All recognized *cis*-acting elements had function for responding to internal and mostly to exogenous stimuli such as light, temperature, wounding and hormones (Table 1). Most of the *cis*-acting elements involved in light responsiveness. According to their functions, the *cis*-acting elements was grouped into seven categories: (A) light-responsive elements (40%), (B) abiotic and biotic-related elements (17%), (C) hormone-responsive elements (13%), (D) development-related elements (12%), (E) promoter-related elements (8%), (F) elements with unknown functions (6%), and (G) site-binding elements (4%). The *cis*-acting elements with a function in light responsiveness had the highest redundancy, but the *cis*-acting elements with a function as site-binding elements had the lowest frequency.

The *cis*-acting elements with annotation of light responsive element, *cis*-acting element involved in light responsiveness, part of a light responsive module and part of a conserved DNA module involved in light responsiveness were all placed

Table 1. The collection of the *cis*-acting elements at the promoter regions of the chloroplast genome of *B. nigra*.

Motifs	Description	Position
CE1	Cis-acting element associated to ABRE, involved in ABA responsiveness	-64 to-56
TGA-box	Auxin-responsive element	-235 to-79
AT-rich sequence	Binding site of AT-rich DNA binding protein (ATBP-1)	-437 to-426
5UTR Py-rich stretch	Cis-acting element conferring high transcription levels	-76 to-27
CE3	Cis-acting element involved in ABA and VP1 responsiveness	-142 to-133
TC-rich repeats	Cis-acting element involved in defense and stress responsiveness	-373 to-262
TATC-box	Cis-acting element involved in gibberellin-responsiveness	-54 to-48
HSE	Cis-acting element involved in heat stress responsiveness	-463 to-445
ACE	Cis-acting element involved in light responsiveness	-225 to-216
LTR	Cis-acting element involved in low-temperature responsiveness	-195 to-190
TCA-element	Cis-acting element involved in salicylic acid responsiveness	-448 to-331
ABRE	Cis-acting element involved in the abscisic acid responsiveness	-137 to-57
ATGCAAAT motif	Cis-acting regulatory element associated to the TGAGTCA motif	-166 to-159
A-box	Cis-acting regulatory element associated with P- and L-box	-233 to-160
ARE	Cis-acting regulatory element essential for the anaerobic induction	-487 to-131
AuxRR-core	Cis-acting regulatory element involved in auxin responsiveness	-139 to-133
Circadian	Cis-acting regulatory element involved in circadian control	-254 to-244
G-box	Cis-acting regulatory element involved in light responsiveness	-252 to-63
RY-element	Cis-acting regulatory element involved in seed-specific regulation	-129 to-58
CGTCA-motif	Cis-acting regulatory element involved in the MeJA-responsiveness	-320 to-315
TGACG-motif	Cis-acting regulatory element involved in the MeJA-responsiveness	-305 to-301
O2-site	Cis-acting regulatory element involved in zein metabolism regulation	-240 to-89
CAT-box	Cis-acting regulatory element related to meristem expression	-190 to-180
CCGTCC-box	Cis-acting regulatory element related to meristem specific activation	-151 to-146
Skn-1_motif	Cis-acting regulatory element required for endosperm expression	-98 to-92
Box E	Cis-element for induction upon fungal elicitation	-197 to-187
GCN4_motif	Cis-regulatory element involved in endosperm expression	-165 to-91
CAAT-box	Common cis-acting element in promoter and enhancer regions	-286 to-69
TATA-box	Core promoter element around -30 of transcription start	-125 to-25

in the group of light-responsive elements. The *cis*-acting elements, which were located in abiotic and biotic-related elements were responsive elements to wounding, pathogens, cold stress, high temperature, anaerobic conditions, and drought stress. In the third group, *cis*-acting elements had the function in response to endogenous hormones including methyl jasmonate, salicylic acid, gibberellin, abscisic acid, and ethylene. The development-related elements included *cis*-acting regulatory element related to meristem expression, *cis*-acting regulatory element related to meristem specific activation, *cis*-acting regulatory element involved in circadian control and MYB binding site involved in flavonoid biosynthetic genes regulation. The last two groups were common regulatory elements at promoter regions such as TATA-box and CAAT-box as well as motifs for binding of DNA-binding proteins (Table 1).

The frequency of the cis-acting elements

Among 339 light-responsive elements, five *cis*-acting elements were predominant, including 47 G box, 28 Sp1, 27 Box 4, and 23 Box I. Totally, 154 abiotic and biotic stress-related elements among, which ARE was detected 31 times, LTR, 21 times, TC-rich repeats, 15 times and Box-W1, 14 times. The total frequency of hormone-responsive elements was 109 and predominant *cis*-acting elements were CGTCA-motif with frequency of 24, TGACG-motif, 22 and ABRE with frequency of 19. Total number of development-related elements was 116 of which, occurrence of Skn-1_motif was 26 times, MBS, 25 times and circadian, 21 times. TATA-box and CAAT-box with the frequency of 92 and 96 respectively, were dominant motifs in the promoter-related elements with total frequency of 217. In the group of elements with unknown functions, total frequency of all *cis*-acting elements was 181. Unnamed elements with frequency of 139 and AAGAA-motif with frequency of 30 were predominant. The latest group - site-binding elements - had total frequency of 11 (Figs. 1, 2).

Co-expression analysis of the plastid genes with abiotic stress-related cis-acting elements

We analyzed protein-protein interaction (PPI) of the genes with ARE, LTR, and other abiotic stress-related *cis*-acting elements. The genes with the elements were atpF, atpI, cemA, clpP, locus_tag = AYB38_cgr001, locus_tag = AYB38_cgr002, locus_tag = AYB38_cgr007, ndhA, ndhB, ndhC, ndhG, ndhH, ndhI, ndhJ, ndhK, petB, psaA, psbA, psbB, psbC, rpl16, rpl2, rpl22, rpoB, rpoC1, rps12, rps12, rps16, rps2, rps7, rps7, trnA-UGC, trnA-UGC, trnG-UCC, trnI-GAU, trnI-GAU, trnI-GAU, ycf1, ycf1, ycf2, ycf2, and ycf3. Among forty-two genes, 29 genes indicated co-expression, 22 genes indicated interactions proved by experimental evidence, and 22 genes indicated co-occurrence (Fig. 3).

GO analysis set all genes into 15 biological processes. Single-organism process with 17 genes and photosynthetic electron transport in photosystem II with three genes had the highest and the lowest observed gene count. The genes were in four KEGG pathways including oxidative phosphorylation (pathway ID:00190), photosynthesis (pathway ID:00195), metabolic pathways (pathway ID:01100), and RNA polymerase (pathway ID:03020). The oxidative phosphorylation pathway had the highest gene count (Fig. 4).

Discussion

In this study, we used simple *in silico* methods to survey *cis*-acting elements at promoter regions of the plastid genes. The results indicated light-responsive elements, hormone-responsive elements, and development-related elements as highly frequent elements through the chloroplast genome. All these element functions have previously proved for the chloroplast through experimental works. In *psbD* a 107 bp segment with three pairs of short, repeated sequences upstream of the core promoter -10/-35 is responsible for regulating of the gene expression at transcriptional level. It has been showed that removing these short sequences had leaded to none-responsiveness to light (Allison and Maliga 1995). The similar results for other plastid genes, *rbcS-3A* and *rbcS-3.6*, have also been reported (Cacchione et al. 1991).

The impact of different types of the plant hormones on chloroplast ultrastructure and pigment contents have been studied (Ouzounidou and Ilias 2005; Haisel et al. 2006; Polanská et al. 2007). In addition, researches have indicated that hormones such as methyl jasmonate, gibberellic acid, abscisic acid, and auxin changes chloroplast gene expression (Yamburenko et al. 2013; Zubo et al. 2011). In *Arabidopsis*, cytokinin signals the chloroplast development and function through the transcription factors ARR1, ARR10, and ARR12 (Rashotte et al. 2006). Brenner et al (2005) identified seven plastid genes (*PETA, PSBG, YCF10, YCF5, MATK, PSBA,* and *PSBI*), which their expression rapidly are induced by cytokinin. High portion of these genes, functions are categorized as transcriptional regulators that signals plastid genes. Some of the plastid genes expression such as most plastid sigma factor genes are regulated in different developmental stages. The plastid sigma factor genes seem to be differentially regulated by circadian rhythms during developmental stages (Oikawa et al. 2000; Ichikawa et al. 2004). The question was that how and which regulatory mechanisms govern changes of sigma factor gene expression. Answer was presence of the important factors AthSig2 and AthSig6. These factors bind to specific segment on the plastid genome, *cis*-acting elements, in response to the developmental and probably environmental

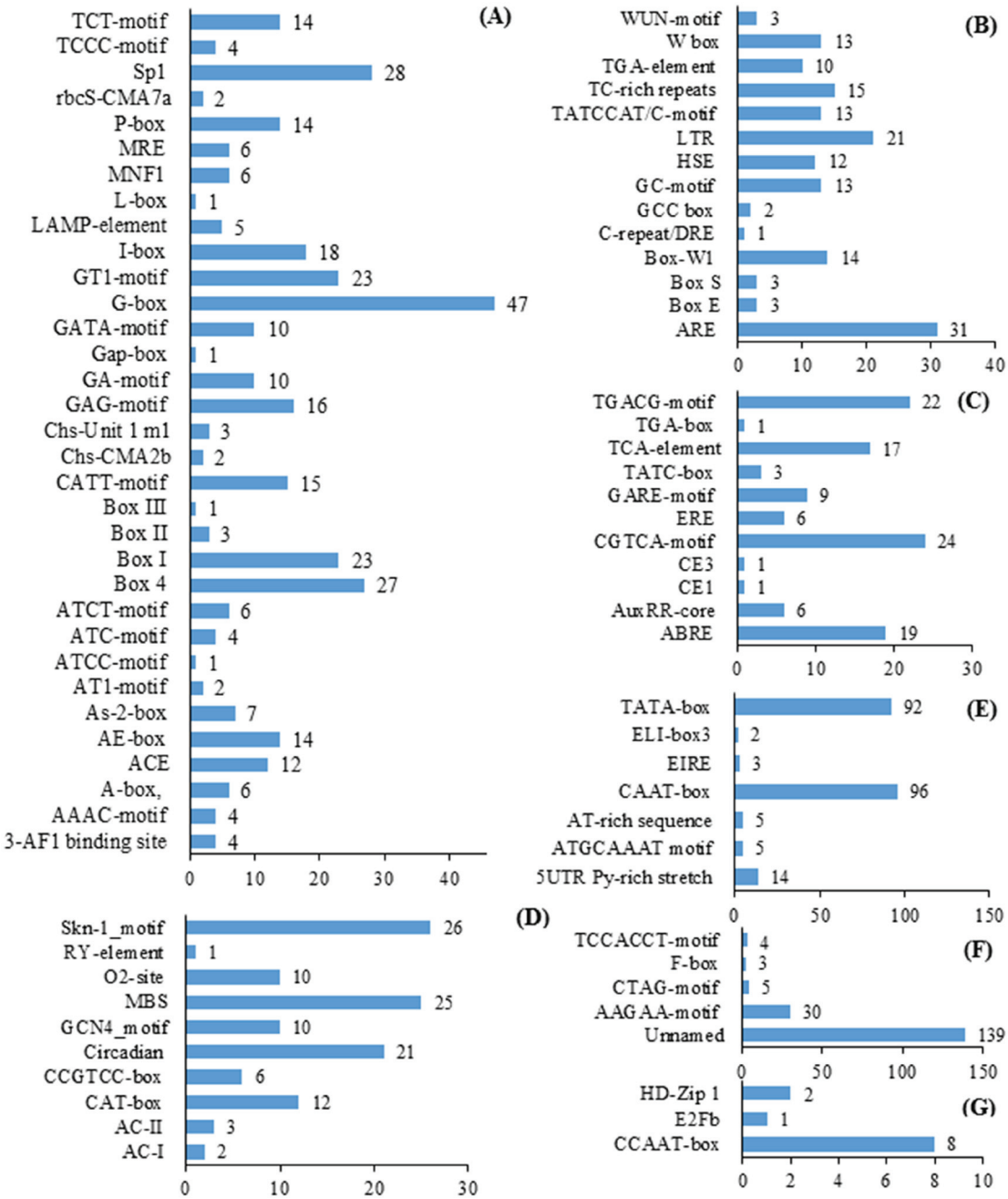

Figure 1. The frequency of the *cis*-acting elements. (A) light-responsive elements, (B) abiotic and biotic-related elements, (C) hormone-responsive elements, (D) development-related elements, (E) promoter-related elements, (F) elements with unknown functions, and (G) site-binding elements.

stresses and changes the affinity of PEP to bind on promoter regions (Lysenko 2007).

All these findings show the importance of *cis*-acting elements in regulatory networks in the chloroplast. Our findings also indicated occurrence of *cis*-acting elements with such functions and high frequency (Table 1; Fig. 2). Comparing our results with the results of experimental works, mentioned above, demonstrate the efficiency of this simple *in silico* approach. Therefore, our new findings could be reliable. However, experimental works are unavoidable requirements for final approval of results. It is found that ARE and LTR were high frequency *cis*-acting elements among abiotic and biotic-

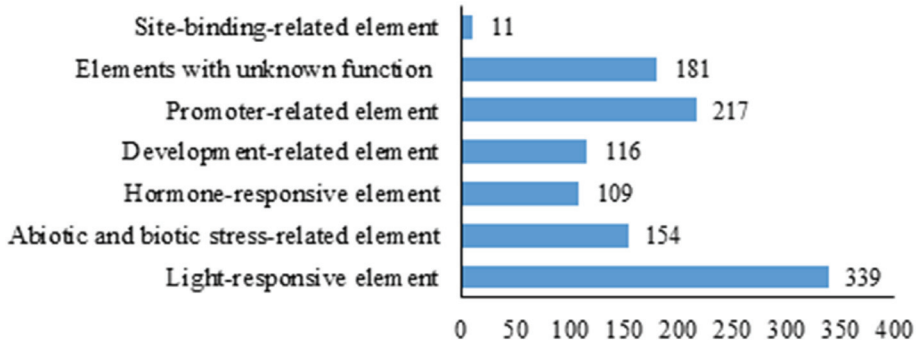

Figure 2. Total frequency of the *cis*-regulatory elements in each functional category. One gene can present in more than one category.

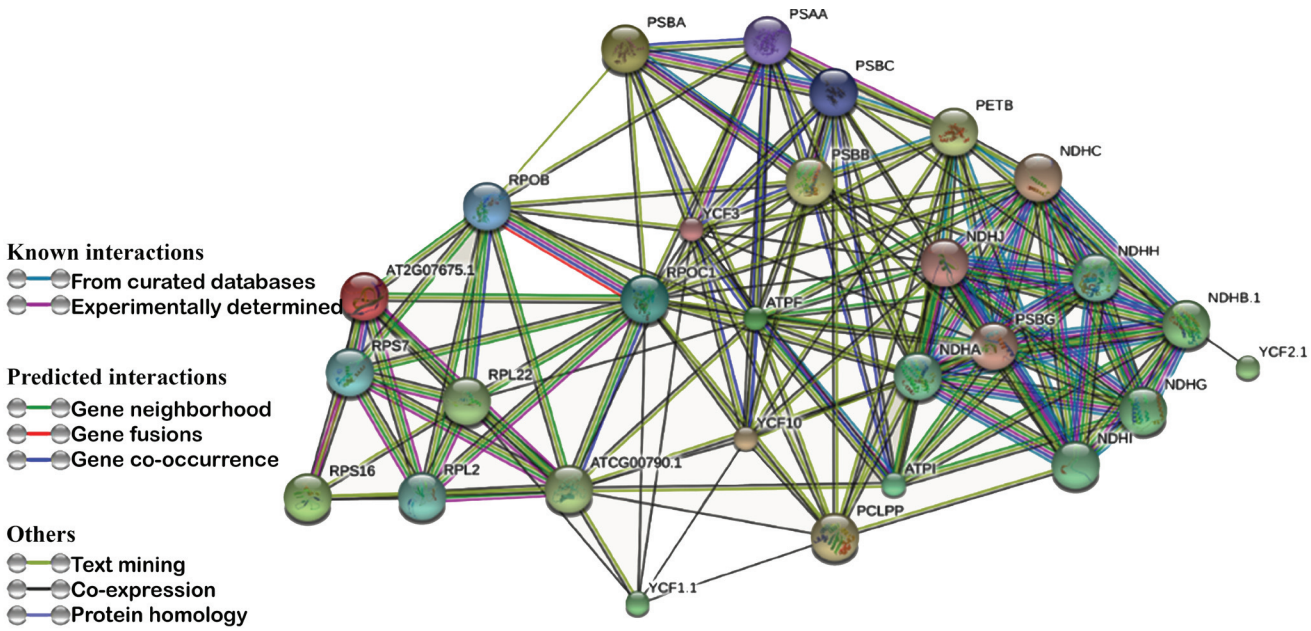

Figure 3. Protein-protein interaction of the plastid genes containing abiotic stress-related *cis*-acting elements. Black lines between nodes indicate co-expression.

related elements (Fig. 1). ARE acts as a *cis*-acting regulatory element essential for anaerobic induction and LTR acts as a *cis*-acting element involved in low temperature responsiveness. Other *cis*-acting elements, involved in dehydration and water deficit, occurred in a low frequency (Table 1). Van Veen et al. (2016) reported upregulation of chloroplast-encoded photosynthesis and redox-related genes under submerged plants and Robinson et al. (2008) reported expression changes of chloroplastic genes under low temperature conditions.

These factors also cause differential expression of many nuclear genes (Licausi et al. 2010; Qi et al. 2012; Shingaki-Wells et al. 2014; Chaudhary and Sharma 2015). The studies show that TFs constitute the major part of abiotic stress-responsive nuclear genes (Chen et al. 2012; Mizoi et al. 2012; Nakashima et al. 2012). On the other hand, over 95% of approximately 3000 proteins in plastid are encoded in the nuclear genome that significant portion of them is TFs for regulating the plastid DNA replication, division, and most of gene expression (Laloi et al. 2006). In addition, domain analysis of nuclear-encoded proteins association with PEP-complex in plastids have demonstrated presence of DNA-binding domains (Melonek et al. 2012). It is inferred that abiotic stresses including anaerobic conditions, low temperature, and water deficit (Table 1), are sensed by nucleus, then the nuclear gene expression profile is changed in response to these stimuli. The part of the nuclear expression-changed with

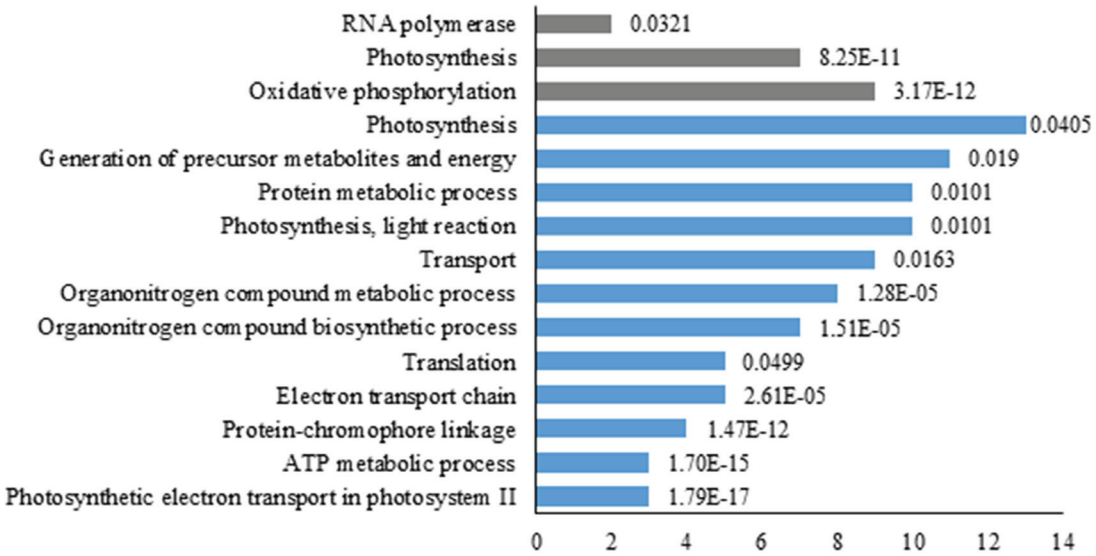

Figure 4. Functional enrichment (Biological Process) analysis of the genes with abiotic stress-related *cis*-acting elements. The bars indicate count in gene set. Data labels indicate false discovery rate. The gray bars indicate the count of genes in KEGG pathways.

TF activity might travel into the chloroplast and influence the plastid gene expression through binding these *cis*-acting elements.

In order, to evaluate the occurrence of abiotic stress-related *cis*-acting elements, we used STRING 10.0 to form protein-protein interaction network. All genes with abiotic stress-related *cis*-acting elements, specifically ARE- and LTR-containing genes, showed good connectivity (Fig. 3). STRING 10.0 calculates connectivity between proteins based on different evidences, in which one of them is co-expression of those protein-encoding genes. Almost all the genes showed co-expression (indicated as black lines between proteins in Fig. 3). Sharma (2015) found that genes co-expressed under osmotic stress share same regulatory motifs. He suggested that co-expressed genes with same motifs are under governing of a specific regulator system. Hudson and Quail (2003) in an attempt to identify promoter motifs in phytochrome A (phyA) -induced and phyA-repressed genes found that G-box and CACGTG-motif were abundant regulatory elements in these two sets of genes. This finding means that TFs induced in respond to an extraordinary stimulus, phyA here, bind to same and conserved sequences among genes responsive to that stimulus. There is another *in silico* and experimental studies such as regulatory elements that control the expression of the *Salmonella csrB* and *csrC* genes (Martínez et al. 2014), involving specific motifs in responsiveness of *Hippocampal* gene expression (Datson et al. 2011), genome-wide identification of DNA binding specificities for the *ApiAP2* family (Campbell et al. 2010), determining *cis*-regulatory elements specific for different types of reactive oxygen species (Petrov et al. 2012), and association of specific *cis*-regulatory elements with abiotic stresses (Bolívar et al. 2014), all indicate same conserved sequences in the upstream region of co-expressed genes in response to a particular stimulus. Therefore, from our results, it could be concluded that co-expression of the genes with abiotic stress-related *cis*-acting elements can represent that they are under same regulatory system in response to the abiotic stresses (Table 1; Fig. 1).

All GO terms were in relation with the functions of chloroplast in higher plants. In our results, single-organism process, single-organism metabolic process, and oxidation-reduction process were parent terms, which were divided into 12-child GO terms (*i.e.* photosynthesis, generation of precursor metabolites and energy, and protein metabolic process; blue bars in Fig. 4). In the case of KEGG pathways, the term metabolic pathway was parent term subdivided into 3-child GO terms including photosynthesis, oxidative phosphorylation, and RNA polymerase pathways (gray bars in Fig. 4). GO enrichment analysis provides terms for representing genes and gene products in living organisms (Shah et al. 2003). We used the term "Biological Process' and "KEGG pathways" to represent gene function and pathway.

According to our GO enrichment analysis (Fig. 4), significant count of genes had photosynthesis function involved in oxidative-phosphorylation pathway. This means that oxidative phosphorylation is more responsive to abiotic stress, taking place in the mitochondria and is linked to photophosphorylation events in the chloroplast (Buchanan et al. 2015). The chloroplast and mitochondria are involved in either the reduction of oxygen or the oxidation of water (Kristiansen et al. 2009). Therefore, the chloroplast and mitochondria are one of the main sources of reactive oxygen species (ROS) pro-

duction sites. Under normal conditions, antioxidant systems scavenger ROSs, prevent cell from being damaged. Under stressful conditions, however, the rate of ROS production is in a level that could harm these organelles and the cell thereupon (Gechev et al. 2006). Plants in order to avoid excessive production of ROSs, in the chloroplast, use variable systems including photorespiration, the cyclic electron flow through PSI or PSII, and the downregulation of 2 PSII quantum yield by the xanthophyll cycle and the proton gradient across the thylakoid membrane (Møller et al. 2007). Our results indicate that the genes involved in oxidative phosphorylation pathways encode two series of proteins: NADH dehydrogenase family protein in the complex I and ATP synthase in complex V. It seems that under abiotic stresses such as salinity, drought, and temperature, water absorption is impaired; a regulatory system controls these genes likely in order to decrease electron flow through regulation of NADH dehydrogenase-encoding genes and producing ATP through regulating ATP synthase-encoding genes. Theoretically, a low level of electron flow and conversion of equivalent equilibrium units in the forms of NADPH and FADH to ATP can be efficient and smart way to restrict electron transferring on oxygen molecules and therefor ROS production. However, this system needs to be confirmed by experimental works.

Conclusions

Based on the role of *cis*-acting elements in regulating gene network in response to diverse types of stimuli, this study demonstrated that the chloroplast genome responds mainly to anaerobic and cold stresses among abiotic stress-responsive genes. In response to abiotic stress, it seems that the chloroplast genome mostly changes the genes involved in oxidative phosphorylation pathway through nuclear-encoded TFs. According to the function of these genes, the regulation is to decrease electron flow on electron transport chain and conversion of NADPH and FADH forms to ATP form in order to lower ROS production. Although, such *in silico* studies have previously been confirmed by experimental works, it is recommended that these genes expression should be studied under abiotic stresses by experimental methods such as RT-PCR.

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