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Research Article

Data-Mining of Barley to Identify Salt Stress Hub Genes, Gene Expression Analysis and Recombinant Plasmid Construction

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Background: Salinity is one of the major abiotic stresses that limit the production and yields of agricultural crops worldwide.

Objectives: In order to identify key barley genes under salinity stress, the available metadata were examined by two methods of Cytoscape and R software. Next, the hub expression of the selected gene was evaluated under different salinity stress treatments and finally, this gene was cloned into cloning and expression vector and recombinant plasmid was made.

Materials and Methods: In this study, we extracted salinity stress tolerant genes from several kinds of literature and also microarray data related to barley under salinity conditions from various datasets. The list of genes related to literature analyzed using string and Cytoscape. The genes from the datasets were first filtered and then the hub genes were identified by Cytoscape and R methods. Next, these hub genes were analyzed for the promoter.

Results: Ten hub genes were selected and their promoters were analyzed, the *cis*-element of which was often *cis*-acting regulatory element involved in the methyl jasmonate -responsiveness, common *cis*-acting element in promoter and enhancer regions and MYBHv1 binding site. Finally, the sedoheptulose-1,7-bisphosp gene (*SBPase*), which had the highest interaction in both gene lists and both types of gene networks, was selected as hub gene. Next, the expression of *SBPase* gene was examined in two variety of Youssef variety (salt tolerant) and Fajr variety (salt sensitive) under salinity stress (NaCl 100mM) at 0 (control), 3, 6, 12 and 24 hours after stress. The results showed that the expression of this gene increased with increasing the duration of stress in both varieties. Comparison of the two varieties showed that the expression of *SBPase* gene in the tolerant genotype was twice as high as sensitive. Finally, *SBPase* gene as a key gene for salinity stress was cloned in both cloning (pTG19) and expression (pBI121) vectors.

Conclusions: According to our results, SBPase gene increased growth and photosynthesis in barley under various abiotic stresses, therefore, over-expression of this gene in barley is recommended to produce plants resistant to abiotic stresses.

Keywords: Cloning, Cytoscape, Gene expression, Microarray, R software, Sedoheptulose bisphosphatase (*SBPase*)

1. Background

Salinity stress is one of the most important abiotic stresses that limit the production and yield of agricultural products worldwide (1). It is reported that about 6% of the world's land (more than 800 million hectares) has been affected by salinity or is exposed to salinization (2, 3). Salinity stress affects plants throughout their life cycle, in terms of molecular, physiological, cellular aspects (4). When plants are exposed to salinity stress, the perception and transmission of stress signals force

the plant to activate stress-related genes, leading to metabolic and physiological changes that adapt the plant to new environmental conditions (5, 6). Today, various genes that increase salinity stress tolerance in plants have been identified and reported to be involved in transcription, signal transduction, ion transport, and metabolic pathways (7, 8). With recent advances in transcriptome analysis technology, a great deal of biological data has become available through online sources that we can combine to obtain information from multiple sources for more reliable results (9-11). Meta-analysis can be used to estimate the expression of differential genes between stress and normal conditions, as well as to identify genes whose products are key molecules in response to stress (9, 12). Meta-analysis is the result of a set of studies that have been systematically collected and evaluated. The degree of reliability and importance of a treatment effect in a metadata analysis is estimated more accurately than in a single study (13). One important method to understand gene function and gene association is co-expression and network analysis (14, 15).

2. Objectives

In order to identify key barley genes under salinity stress, the available metadata were examined by two methods of Cytoscape and R software. Next, the hub expression of the selected gene was evaluated under different salinity stress treatments and finally, this gene was cloned into cloning and expression vector and recombinant plasmid was made.

3. Materials and Methods

3.1. Identification and Screening of Salinity Tolerant Genes

3.1.1. Using Literature and Text Mining

Barley salt-tolerant genes were extracted from almost all published literature and articles in reputable journals at the time of research. Gene sequences were obtained from the NCBI site. Then, the most similar sequence accession of all genes was obtained for Arabidopsis (as a model plant). Next, their UniGene codes were determined using the NCBI or UniProt database and recorded. The STRING database (https://string-db.org/) (16) was used to determine the interaction of proteins, in which the UniGene codes of Arabidopsis were introduced into the program, then the node1 and node2 data were saved and used to plot the gene network by Cytoscape software (version 3.9.1) (17). The gene network was plotted using the CytoHubba plugin (version 0.1) (18), based on three MNC, Degree, and Closeness algorithms. Finally, the networks obtained from the three algorithms were compared and the genes with the highest interaction were selected.

3.1.2. Using GEO Datasets of NCBI

Transcriptome profiles of barley (*Hordeum vulgare*) under salt stress were obtained from GEO datasets, accession numbers of GSE3097, GSE5605, GSE6325, GSE41517, and GSE41518. The data were analyzed and the expression ratio of salt treated genes to control genes (without stress) was calculated and determined based on log FC. Then, the probes with log FC above +2 were selected and saved.

3.1.2.1. Determination of Co-Expression Genes by R Software

Data analysis was done using R, *GeneNet* package (version 1.2.16) (19). First, co-expression gene modules were determined for all genes, within each module were placed genes with similar gene expression. Second, the co-expression diagrams and gene networks were plotted for each module. Finally, the networks were compared and the genes with the high replication and interaction were selected.

3.1.2.2. Determination of Protein Interaction and Identification of Hub Genes

The probe sequences were received from the PLEXdb site (http://www.plexdb.org/) (20) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (21) was performed on them. Then, the most similar sequence accession was obtained and using the TAIR database (https://www.arabidopsis.org/) (22), Arabidopsis UniGene code was determined and recorded. This operation was conducted for all probes. As in the first method (literature and text mining), protein interaction was performed by String program and gene network analysis by Cytoscape program.

3.2. Promoter Analysis of High-Interaction Genes

The promoter sequence of selected genes was extracted from NCBI and was used for *cis*-element identification. To find the regulatory elements in the promoter sequence, the web-based PLANTCARE

database (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (23) was used.

3.3. Hub Gene Selection and Primer Design

At this point, the gene that was common to all sources (literature and datasets: gene network and co-expression network) was selected as the hub gene. Because the selected gene was a predicted gene in barley (NCBI ID: AK363491), so complete sequence of this gene was obtained from rice (NCBI ID: AK119209). Next, the sequences aligned using T-Coffee server (https://tcoffee.crg.eu/) (24). From the conserved areas, the end of 5' and 3', degenerate primer design was performed. Homodimer, heterodimer, stem-loop, GC percent, and TM temperature were measured using Vector NTI Advance (version 11.0) (25) and Oligo software (version 7.60) (26). The primers were then synthesized at Bioneer Company of South Korea.

3.4. Plant Material and Cultivation

Seeds of Youssef variety (salt tolerant) and Fajr variety (salt sensitive) of barley were obtained from seed and plant improvement institute in Karaj, Iran. Seed germination was performed at 25 °C, and under 100% humidity and dark conditions on a wet filter paper. After germination, they were transferred to containers (with water) and in the seedling stage (two leaves), salt stress was applied (27).

3.5. Salt Stress Treatment and Gene Expression Analysis

3.5.1. Salt Stress Treatment

To apply salinity stress, a 100 mM solution of NaCl was prepared (28-30). Two-leaf seedlings of two varieties were then placed in NaCl-containing containers for 0 (control), 3, 6, 12 and 24 hours. After treatment, seedlings were separated from the containers and transferred to the freezer -80 °C for RNA extraction.

3.5.2. Gene Expression Analysis

Total RNA was isolated from fresh leaves using a total RNA kit (RB1001, RNA, Iran), and DNase I enzyme (RB125A, RNA, Iran) was used to remove genomic DNA contamination. Then, the first-strand cDNA was synthesized (RB125A, RNA, Iran) based on the manufacturer's instructions. qPCR with *SBPase* and *HKP* (House-keeping gene: *Actin* gene) primers was performed to evaluate the performance and specificity as well as the setting of the primers. qPCR amplification was done with an Applied Biosystems™ StepOne™ qPCR System (Applied Biosystems, CA, USA) using SYBR® Green qPCR Master Mix (RB120, RNA, Iran). PCR reactions were provided in a final volume of 20 µL containing 1 µL of synthesized cDNA, 10 µL 2×SYBR, 2µL of each forward and reverse primers, and 7 µL distilled water. The reactions included a primary step of 95 °C for 20 secs, then 35 cycles at 95 °C (20 sec) for denaturation, and 52 °C (45 sec) for primer annealing and 72 °C (40 sec) for extension. Subsequently, the melting curves was plotted from range 52 °C to 95 °C in each reaction to check the specificity of the amplicons. Three biological replicates of samples (fresh leaves) were employed for the qRT-PCR analysis, and three technical replicates were investigated for each biological replicate. Efficiency and Ct values were computed using the LinRegPCR quantitative PCR data analysis program (version 11.0) (31), and relative expression values were analysed by REST method (version 2009) (32) as the following formula:

$$Gene\ expression\ ratio = \frac{(E_{GOI})^{\Delta CT\ GOI}}{(E_{HKG})^{\Delta CT\ HKG}}$$

The "E" in the equation refers to the primer efficiency.

3.6. Construction of Recombinant Plasmid

3.6.1. DNA Extraction

Due to the fact that our gene was a single exon, DNA was extracted to isolate the gene. The genomic DNA was extracted from 300 mg of each sample by CTAB-based method according to Gabriadze *et al*. (33).

3.6.2. Cloning of Candidate Gene

Candidate gene was amplified using related forward and reverse primers. PCR was carried out by Taq polymerase using the following conditions: Initial denaturation (94 °C for 5 min) and then 35 cycles of cycle denaturation (94 °C for 1 min), annealing (50 °C for 1 min) and extension (72 °C for 5 min). PCR products were cloned into pTG-19 vector (Both pTG-19 vector and the PCR product have TA end), and then the recipient plasmid was transformed into *Escherichia coli* DH5α competent cell. The bacteria were growth in LB agar (Luria-Bertani) containing Ampicillin (50µg. mL^{-1}) at 37 °C for 16 hours. The candidate gene was amplified by PTG19 plasmid proliferation inside the *E. coli* bacteria. To confirm the cloning, first the plasmid

was extracted from the bacteria and then it was digested with BamHI enzyme.

Next, Sequences of BamHI and SacI site added to 5' ends of *SBPase* forward and reverse primers, respectively. After DNA plasmid extraction from colonies, PCR was performed using mentioned primers (Forward and reverse primers have a digest site BamHI and SacI at the 5'ends). Then, the amplified fragment was cloned into the plant expression vector pBI121. For this purpose, amplified fragment and $pBI121^{GUS-9}$ vector were double digested with BamHI and SacI. The Target fragment was ligated into $pBI121^{GUS-9}$ and transformed into *E. coli* DH5α. Then, the bacteria were growth in LB agar containing Kanamycin (50 $\mu g.mL^{-1}$) at 37 °C for 16 hours. To confirm cloning, the plasmid was double digested with enzyme BamHI and SacI.

4. Results

In this study, 22841 probes were obtained from datasets for different tissues and levels of salinity stress. Of these, 2212 genes had Log FC = ±2.5. In addition, 368 genes responding to salinity stress reported in the literature were selected.

4.1. Selection the Hub Gene

First, the genes extracted from the literatures were examined for protein interaction (**Fig. 1C-**Literature). Then the interacting proteins were entered into Cytoscape software and the hub genes were identified. According to **Figure1-B1**, the hub genes of the Closeness algorithm consisted of *HSP70*, *HSP70-15*, *P5CS1*, *CPN60B*, *GS2*, *UBQ14*, *SBPase*, *UBQ10*, At5g06290 and AT3G60750. Whereas, the hub genes of the Degree algorithm (**Fig. 1-C1**) consisted of *RPS1*, *ATPD*, *CPN60B*, *SBPase*, *RCA*, *GS2*, *UBQ14*, *UBQ10*, *P5CS1* and At5g06290, and the hub genes of the MNC algorithm (**Fig. 1-D1**) consisted of *CPN60B*, *RPL12-C*, *SBPase*, *GS2*, *ATPD*, *RCA*, *UBQ10*, *UBQ14*, AT3G60750 and At5g06290. Comparison of algorithms showed that genes *SBPase*, *CS2*, *CPN60B*, *UBQ14*, *UBQ10* and At5g06290 were similar between the three algorithms. Second, the protein interaction of the genes derived from the dataset was determined (**Fig. 1-A2**), then the gene network was drawn for the hub genes with three different algorithms. The Closeness algorithm (**Fig. 1-B2**) included *HSP60*, *SBPase*, *HSP90.1*, *GAPA-2*, *P5CS1*, *HSP70*, *HSP81-2*, *CPN60B*, *UBQ10* and *EMB3113* genes, the Degree algorithm (**Fig. 1-C2**) included *HSP70*, *HSP60*, *SBPase*, *P5CS1*, *ACT7*, *UBQ10*, AT5G66180, AT3G57490, AT1G74050 and AT1G70600 genes, and the MNC algorithm (**Fig. 1-D2**) included *HSP60*, *HSP90.1*, *GAPA- 2*, *UBQ10*, *SBPase*, *HSP70*, *HSP81-2*, *EMB3113*, AT1G06560 and AT3G57490. According to the results of the gene network in the three algorithms, the *HSP60*, *SBPase*, *HSP70* and *UBQ10* genes were common between the algorithms. Comparison of common genes between the three algorithms related to Figures 1-Literature and 1-Datasets showed that between the networks of two gene groups, two genes *SBPase* and *UBQ10* are common.

For the genes derived from the datasets, in addition to protein interaction and gene network, co-expression genes were also determined. For this purpose, first co-expression modules in the gene set were determined and then for selected modules, co-expression diagrams and co-expression network were drawn. The gene sets were grouped into 23 modules (**Supplementary 1**).

From these modules, 4 modules that had the most interaction were selected and the co-expression diagram were drawn for them. Module 5 contained 61 genes that the expression of these genes was maximal (8.5) in sample A5 (MeJA: Methyl jasmonate) (**Supplementary 2A**). Module 7 consisted of 29 genes and the genes in this module showed the highest expression (4.5 and 4) in samples A7 and A9 (Maythorpe Cultivar-Shoot and cultivar Morex after 8 hours' stress, respectively) (**Supplementary 2B**). Modules 8 and 11 also contained 10 genes that in module 8, sample A5 (Methyl jasmonate treatment) showed the highest expression (6) and in module 11, samples A4 (Golden promise root cultivar) and A6 (treatment with MeJA and salinity stress) had the highest expression (3 and 4.5, respectively) (Sup. 2C and 2D). Next, a gene network was drawn for these 4 modules.

The gene network identifies hub genes and their interactions in each module (**Fig. 2**). As shown in **Figure 2A**, the hub genes in Module 5 include AT5G43220, *HSP90.1*, *TRI*, *PAL2*, *DOX1* and At5g46330. The hub genes related to Module 7 (**Fig. 2B**) include *4CL1*, *RCA*, *BOA*, *AGKbox*, *DCP2* and *ANNAT1*, and the hub genes in Module 8 (**Fig. 2C**) include AT3G13560, AT4G36180, *ABCG38*, AT3G60340 and *ARF19*. Module 11 (**Fig. 2D**) also contained *SBPase*, AT1G33440, *CKA3* and *1-Apr* genes. The results showed that *SBPase* gene is common between Cytoscape and R gene networks and is known as a key gene in both methods.

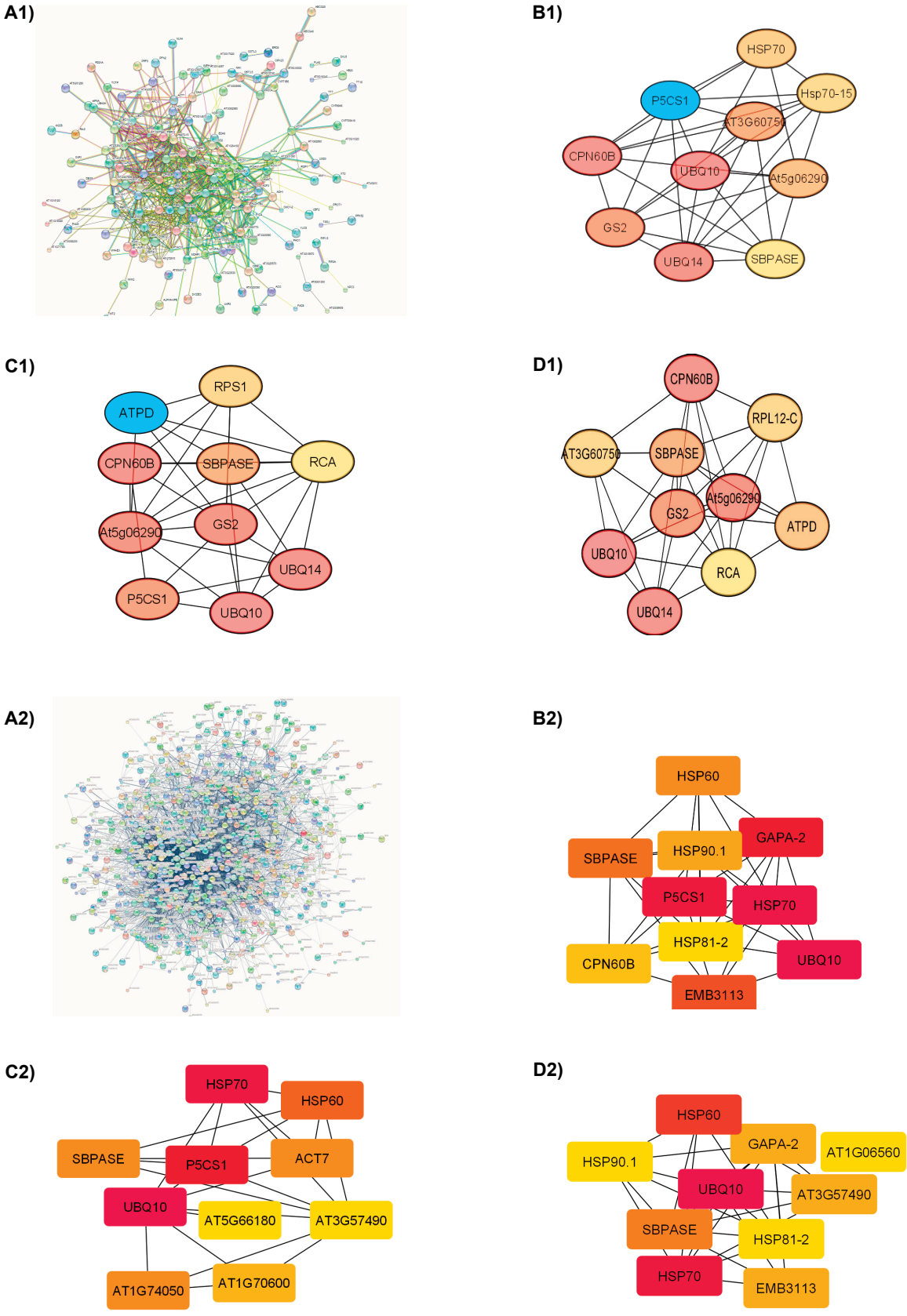

Figure1. Protein interaction and gene network in genes extracted from literatures (**1**) and datasets (**2**). **A**) proteins interaction, **B**) gene network based on closeness algorithm, **C**) gene network based on degree algorithm, **D**) gene network based on MNC algorithm.

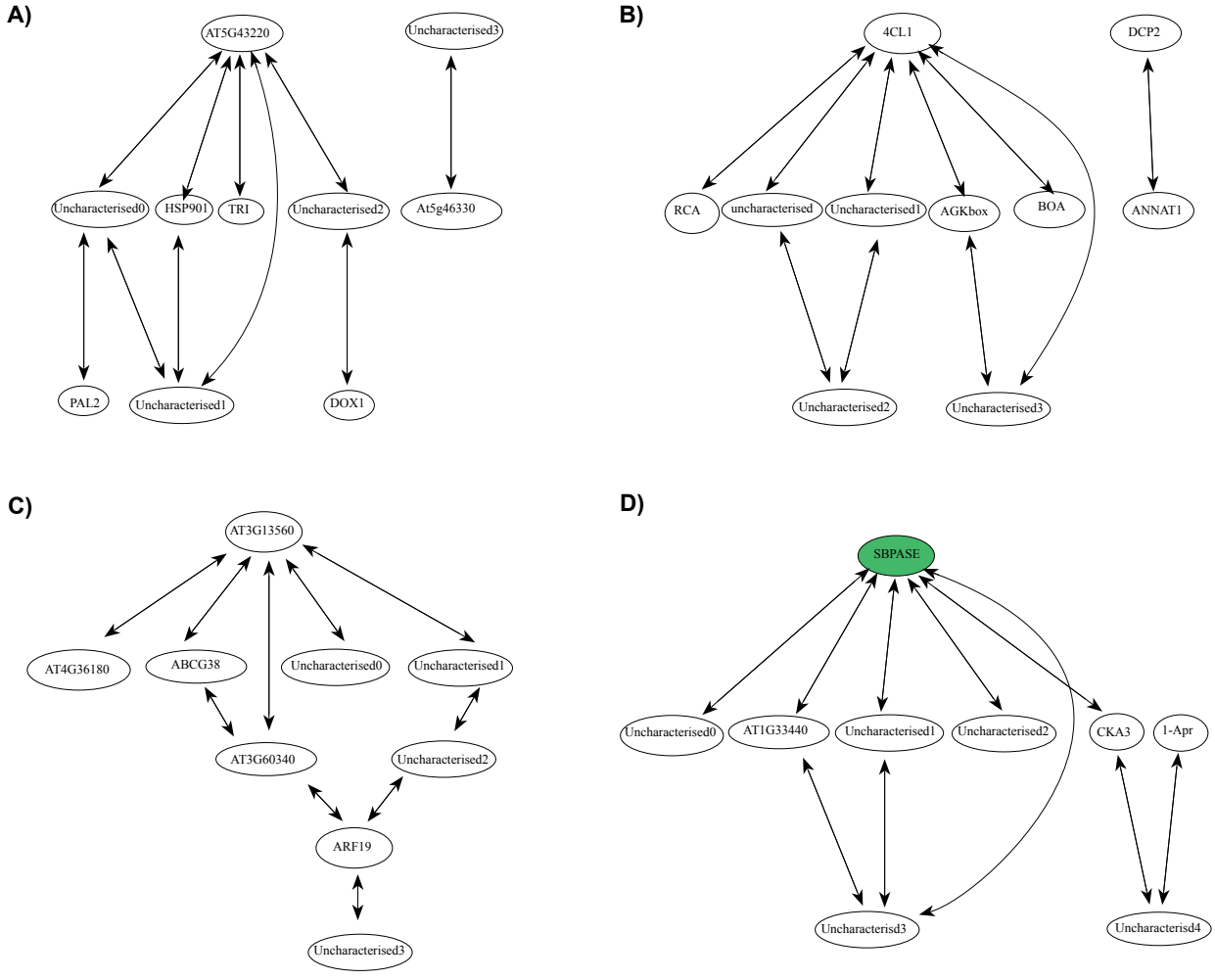

Figure 2. Gene network for genes within each module. **A**) Module5, **B**) Module7, **C**) Module8, **D**) Module11.

4.2. Promoter Analysis of Selective Genes

We collected hub genes from both gene networks (Cytoscape and R) and analyzed their promoters. As shown in **Table** 1, most genes have *cis*-elements of *cis*-acting regulatory element involved in the MeJA-responsiveness, common *cis*-acting element in promoter and enhancer regions and MYBHv1 binding site (34).

4.3. Gene Expression Analysis under Salt Stress

A comparison of gene expression between tolerant (Youssef) and sensitive (Fajr) varieties was performed at 4 salinity levels with three replications. According to **Figure 3**, the expression of *SBPase* gene in the control treatment (without salt stress) was not significantly different from the *HKP* gene in both varieties and its expression was almost equal to the *HKP* gene. In salinity stress treatments, with increasing the duration of stress, *SBPase* gene expression increased in both varieties. As shown in **Figure 3**, the expression of this gene in the tolerant variety is almost twice that of the sensitive variety. The highest expression of *SBPase* gene in tolerant variety was related to 24 hours of salinity stress treatment (6.2) and in sensitive variety was related to 12 hours of salinity stress treatment (3.2). The lowest expression of this gene in both tolerant and sensitive varieties was related to control treatment (1.12 and 0.96), respectively.

4.4. Cloning of Selected Hub Gene to Vectors

Among the identified hub genes, the *SBPase* gene was selected for cloning due to its presence in all gene networks and also high gene expression. First, the *SBPase* gene was amplified with the corresponding forward and reverse primers (**Fig. 4A**).

Table1. Motifs and cis elements function of selective gene promoters.

Gene name	motif	Function
Gapa	5'-CAAT-3' 5'-GCTCA-3' 5'-TCACG-3'	cis-acting regulatory element involved in the MeJA-responsiveness (30)
UBQ10	5'-CAAT-3' 5'-CCAAT-3' 5'-CGTCA-3' 5'-TGACG-3'	common cis-acting element in promoter and enhancer regions MYBHv1 binding site cis-acting regulatory element involved in the MeJA-responsiveness (30)
CPN60B	5'-CAAT-3' 5'-CCAAT-3'	common cis-acting element in promoter and enhancer regions MYBHv1 binding site
P5CS1	5'-CGTCA-3' 5'-CCAAT-3' 5'-CGTCA-3' 5'-LTR-3' 5'-TGACG-3' 5'-CAAT-3'	cis-acting regulatory element involved in the MeJA-responsiveness MYBHv1 binding site cis-acting regulatory element involved in the MeJA-responsiveness cis-acting element involved in low-temperature responsiveness common cis-acting element in promoter and enhancer regions (30)
AT3G60750	5'-CGTCA-3' 5'-TCACG-3' 5'-CAAT-3'	cis-acting regulatory element involved in the MeJA-responsiveness common cis-acting element in promoter and enhancer regions (30)
At5g06290	5'-CGTCA-3' 5'-CCAAT-3' 5'-GAG-3' 5'-CAAT-3'	cis-acting regulatory element involved in the MeJA-responsiveness MYBHv1 binding site part of a light responsive element common cis-acting element in promoter and enhancer regions (30)
HSP70	5'-CCAAT-3' 5'-CGTCA-3' 5'-TGACG-3' 5'-CAAT-3'	MYBHv1 binding site cis-acting regulatory element involved in the MeJA-responsiveness common cis-acting element in promoter and enhancer regions (30)
HSP70-15	5'-CGTCA-3' 5'-TGACG-3' 5'-CAAT-3'	cis-acting regulatory element involved in the MeJA-responsiveness common cis-acting element in promoter and enhancer regions (30)
SBPASE	5'-ABRE-3' 5'-CAAT-3'	cis-acting element involved in the abscisic acid responsiveness common cis-acting element in promoter and enhancer regions (30)
EMB3113	5'-GAG-3' 5'-CGTCA-3' 5'-LTR-3' 5'-TGACG-3' 5'-CAAT-3'	part of a light responsive element cis-acting regulatory element involved in the MeJA-responsiveness cis-acting element involved in low-temperature responsiveness common cis-acting element in promoter and enhancer regions (30)

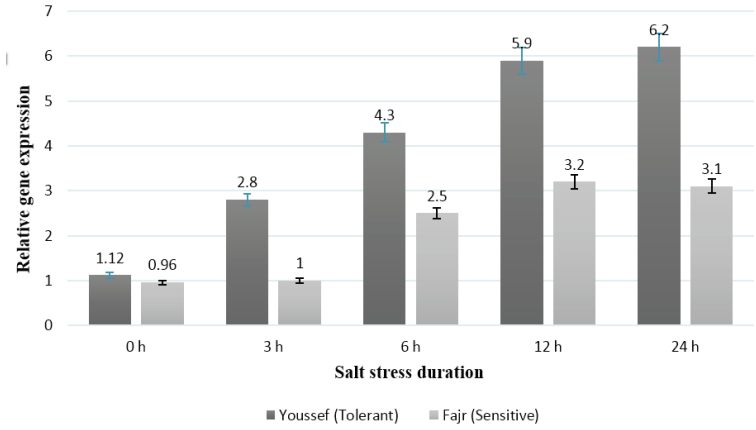

Figure 3. Gene expression of *SBPase* in two varieties of Youssef and Fajr under 4 levels of salt stress compared to *HKP* gene. The number of biological and technical repetitions in both varieties and both genes (*SBPase* and *HKP*) was three.

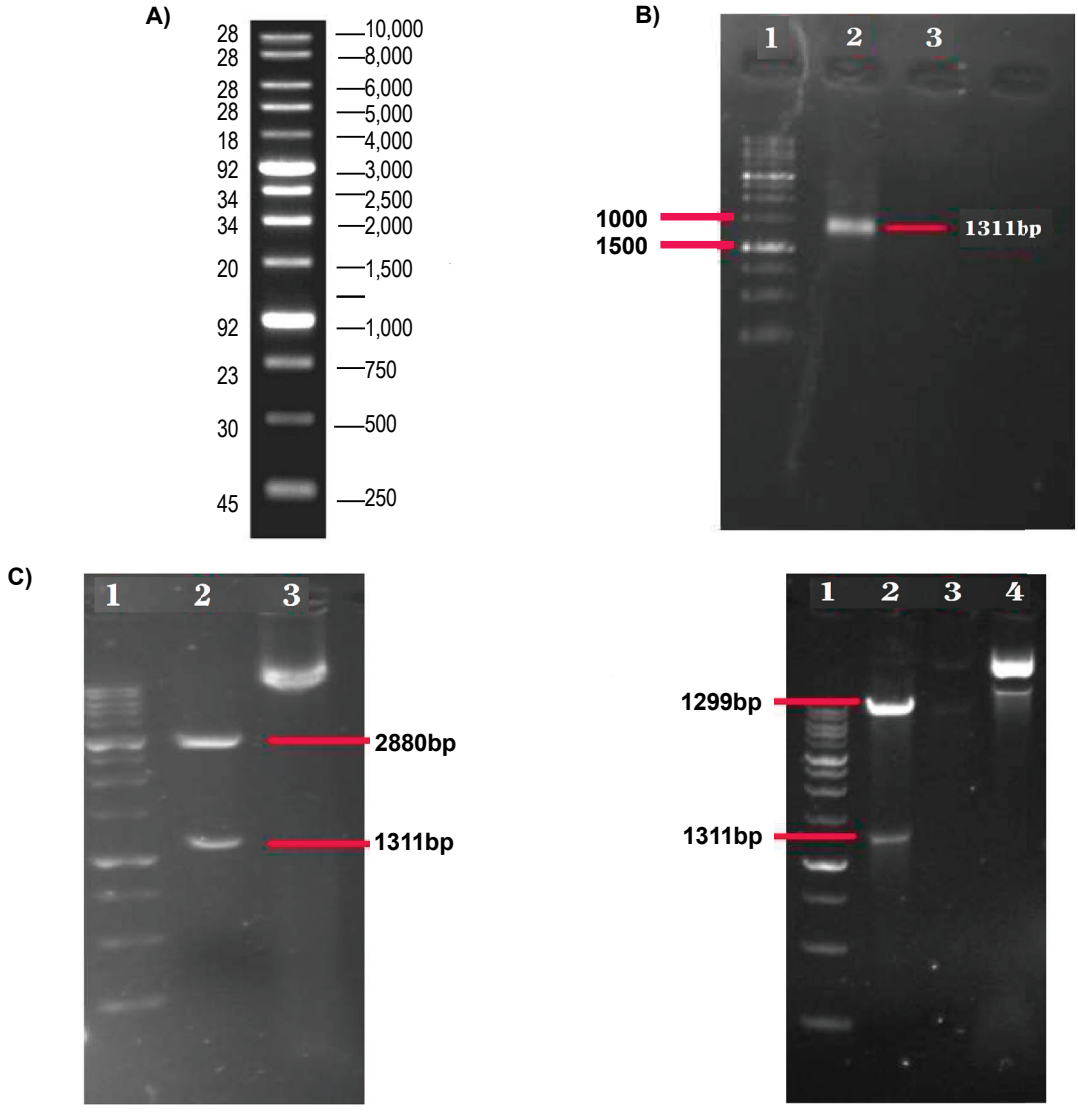

Figure 4. Cloning of SBPase gene in plant expression vector. **A**) DNA ladder, **B**) 1= ladder, 2= PCR product, 3= Negative control, **C**) Digestion of pGT-19 Vector by BamHI enzyme. 1= ladder, 2= digested vector, 3= undigested vector, **D**) Digestion of pBI121GUS-9 Vector by BamH1 and *Sac*I enzymes 1= ladder, 2= digested pBI121GUS-9 vector, 3= digested Non-gene vector, 4= undigested vector

According to Figure 4a, the length of our amplified fragment is 1311bp. *SBPase* gene was then ligated with the cloning vector pTG19 for amplify the DNA fragment of interest (35) (**Fig. 4B**).

To confirm the ligation of the *SBPase* gene with the pTG19 vector, we digested the recombinant plasmid with the BamHI enzyme, which results in two bands on electrophoresis gel, including the 2880 bp fragment belonging to the vector and the 1311 bp fragment belonging to the *SBPase* gene. Next, the gene was cloned into the pBI121 plant expression vector (**Fig. 4C**). To confirm the gene cloning in pBI121 vector, we used two enzymes, BamHI and SacI. Digest results consisted of two fragments with lengths of 12994bp and 1311bp, which were related to pBI121 vector and *SBPase* gene, respectively (**Fig. 4D**).

The schematic view of vectors pTG19 and pBI121 map along with *SBPase* gene inserted in them and the insertion site is presented in **Figure 5**.

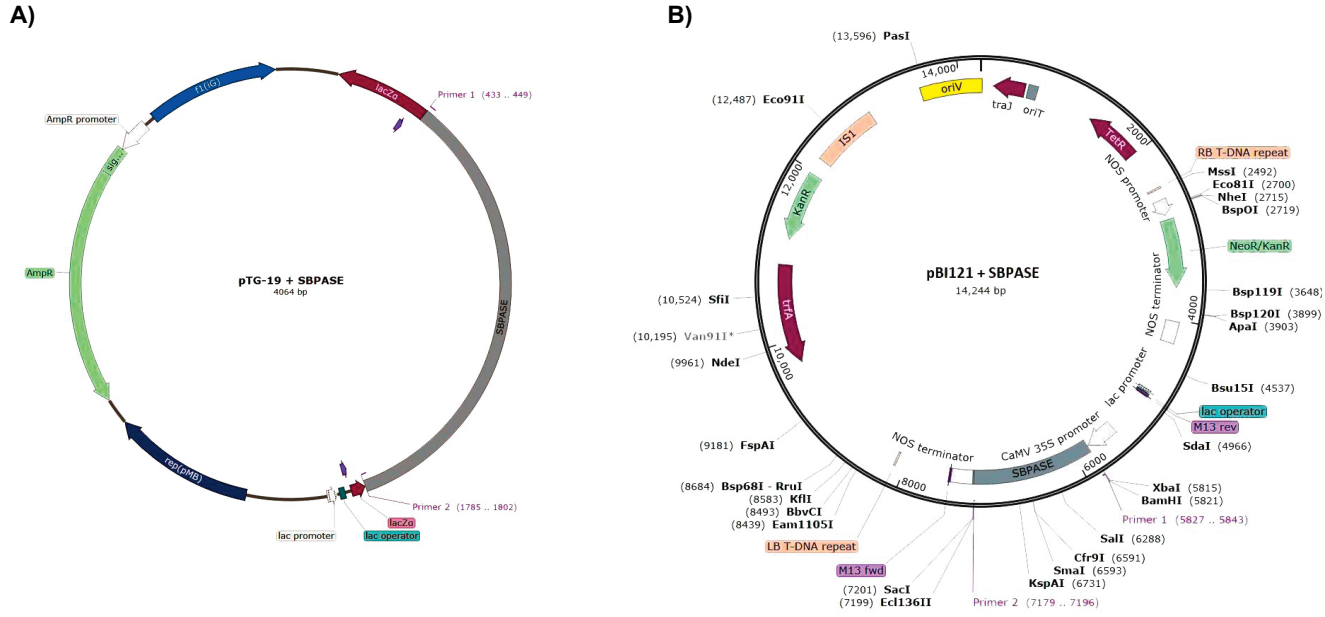

Figure 5. Cloning (pTG19) and expression (pBI121) vectors with SBPase gene, insertion site, and forward and reverse primers. A) pTG-19 vector + SBPase gene (4064 bp), **B**) pBI121 vector + SBPase gene (14244 bp).

5. Discussion

Abiotic factors are the main yield-limiting agents for plants (36). Temperature, drought, salinity, and heavy metal stress affect the growth and yield of plants (37). The growth and production of plants are influenced by a response generated by a group of genes by changing their expression patterns. Therefore, identification of genes responding to abiotic stresses is essential to understanding the response mechanisms in plants (38). With the development of high-throughput sequencing and various omics technologies, large amounts of sequence datasets and global changes in gene expressions have been reported. A large number of genes responding to salinity stress have been isolated and their function has been identified, including photosynthesis genes and synthetic enzymes that regulate metabolism (39-43). The function analysis of stress-related genes also is an essential tool to understand the molecular mechanisms of stress tolerance and the responses of higher plants to stress, as well as, improve the plant's stress tolerance by gene manipulation (44).

According to the results obtained in this study, the hub genes involved in salinity stress tolerance include *Gapa*, *UBQ10*, *CPN60B*, *P5CS1*, AT3G60750, At5g06290, *HSP70*, *SBPase* and *EMB3113*. As the promoter analysis showed, these genes are often involved in *cis*-acting regulatory element involved in the MeJA-responsiveness, MYBHv1 binding site, and common *cis*-acting element in promoter and enhancer regions. Among these hub genes, gene *SBPase* which was common in datasets (Cytoscape and R) and literatures was selected as the key gene for transfer to sensitive plants.

The enzyme *SBPase* (sedoheptulose-1, 7-bisphosphatase) functions in the regenerative phase of the Calvin cycle. This enzyme is important in the Calvin cycle because it is the point where carbon gets committed to regeneration of the acceptor molecule RuBP (Ribulose 1,5-bisphosphate). Research has shown that *SBPase* plays an important role in regulating carbon flow in the Calvin cycle, and reduced expression in transgenic plants leads to reduced photosynthesis in the plant (45, 46). *SBPase* gene causes negative osmosis pressure by increasing the rate of photosynthesis, sucrose and starch accumulation inside the cells, this preserves the cell water (47). Rubisco regenerative capacity also responds linearly to reduce *SBPase* activity in antisense *SBPase* plants (48). Researchers showed that overexpression of *SBPase* under salinity stress increased photosynthesis and growth in transgenic rice plants (49). The results indicated that under salinity stress, *SBPase* maintains

RuBP carboxylase-oxygenase activity by further regeneration of the Rubisco receptor molecule in the soluble stroma. It also prevents the degradation of Rubisco-activase from soluble stroma to the thylakoid membrane, thus increasing photosynthesis under salinity stress. Results suggested that overexpression of *SBPase* was an effective method for increasing salt tolerance in rice. Ding *et al*. (2016) reported that changing *SBPase* expression affects growth, photosynthetic capacity, and cold stress tolerance in transgenic tomato plants. They suggested that higher level of *SBPase* activity gives an advantage to photosynthesis, growth and chilling tolerance in tomato plants (50). Driever *et al*. (2017) also reported that increased *SBPase* activity improved photosynthesis and grain yield of wheat in greenhouse conditions. Transgenic plants with increased *SBPase* protein levels and activity were grown under greenhouse conditions (51). The results showed that photosynthesis, total biomass, and dry seed yield increased in these plants. It has been reported that *SBPase* gene increases the amount of biomass in maize and sorghum (52). When the *SBPase* gene is overexpressed, the yield of tobacco and soybean is increasing in the farm (53). The yield of maize has also been improved by overexpressing the *SBPase* gene (54). When the *SBPase* gene was overexpressed in Afzal cultivar, the abiotic stress tolerance of this cultivar was more than that of the sensitive cultivar of L-527 (55). Thus, the activity of the *SBPase* gene in the c3 plants increases carbon assimilation, resulting in tolerance of these plants to abiotic stresses, particularly salinity (56). In the sensitive tomato plant, there is a *SBPase* gene. However, the increase of H_2O_2 in parallel with decrease of *SBPase* activity is due to the suppressing effect of reactive oxygen. Nevertheless, in the transgenic plants in which *SBPase* is overexpressed, there has been an increase in carbon fixation and carbohydrate accumulation. Therefore, transgenic tomatoes were more tolerant to chilling stress than other sensitive varieties (57). There have been few reports on the role of the overexpression of *SBPase*, and whether it is capable of enhancing growth and photosynthesis during salt stress in important crops such as rice but no research has been done on barley.

6. Conclusion

Our results indicated that *SBPase* gene increased growth and photosynthesis in barley under various abiotic stresses. Due to the fact that in this paper we used both the results presented in the literatures and all the microarray data available on the NCBI site, and also because we used two different methods of analysis (Cytoscape and R) to find the hub genes, so the results obtained in this study are extremely strong for the presentation of hub genes and the use of these genes to increase tolerance to salinity stress in barley is recommended.

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