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CLONING AND CHARACTERIZATION OF HEAT SHOCK FACTOR (BVHSF) FROM SUGAR BEET (*BETA VULGARIS*)

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Abstract

This study focused on influence of under environmental stresses on heat shock factor gene expression in sugar beet (*Beta vulgaris* L.) under greenhouse condition. qPCR analysis of gene expression in sugar beet was used to determine changes in gene expression after application of 0%, 3%, 5% and 7% (w/v) PEG for one month in Hoagland solution. Glutamine synthetize is housekeeping gene was used as endogenous control, while the target was heat stress transcription factor *BvHSF* gene showed a significantly up regulation expression of under drought stress. The obtained results indicated that qRT-PCR protocol was effectual to expression analysis for *BvHSF* under water stress. The gene was cloned and bioinformatically analyzed and was further transformed into Arabidopsis thaliana plants for further studies. Therefore, this research could help in deep understating of plant response to stress and help to improve plant breeding.

Keywords: *Beta vulgaris* L., qPCR, heat shock factor, housekeeping gene, *Arabidopsis thaliana*

Introduction

Sugar beet (*Beta vulgaris*) is the second largest cultivated sugar producing crop after sugarcane worldwide, where about to 30% of the world sugar production is produced from sugar beet (Sen and Alikamanoglu, 2012 & Iqbal and Saleem, 2015). About 4.8 million hectares of sugar beet were cultivated worldwide, which produce around 300 million tons of sugar. In Egypt, sugar production is estimated to be about 2.74 thousand tons annually, from which, 1.5 thousand tons comes from sugar beets (Faostat, 2017).

Like most commercially cultivated crops, sugar beet is negatively affected by environmental stress conditions which negatively affects photosynthesis efficiency, thus affecting plant development and accumulation of sucrose in roots (Ober and Rajabi 2010). In general, abiotic stresses, as in extreme temperatures, drought and salinity are very much overlapping making it difficult to separate their negative effects on performance of most economically important crops in the field (Pidgeon *et al*., 2006 & Richter *et al*., 2006).

High temperatures and dehydration-induced stresses tend to have synergistic influences on plants, as they cause an imbalance in plant water content, where evaporation exceed water intake, resulting in welting of plants in the field (Kumar *et al*., 2004). Although sugar beet is considered among drought-moderately tolerant species, yet sever cycles of drought, especially during early germination stages, has significant effects on growth parameters in general (Monirifar *et al*., 2004), leading to dramatic decrease in yield (Sen and Alikamanoglu, 2012 & Romano *et al*., 2013). Currently, global warming phenomenon is affecting productivity of major crops worldwide, and it is no surprise that dissecting plants response to changes happening at surrounding environment is of a great importance (DaMatta *et al*., 2003)

Under abiotic stress environments, plants are likely to undergo changes at molecular, biochemical, morphological, and physiological levels to accommodate changes in the surrounding environment (DaMatta *et al*., 2003). For example, at the molecular level, different classes of genes and molecules tend to increase/decrease their levels within a given plant cell in response to changes in the surrounding environment. Ca-ions are well identified as a plant signaling molecules involve in cell progression for response to abiotic stress, it is one of the first changes that tend to occur in a given plant cell is the changes in free Ca ions in the cytosol, also known as Ca-signature, there is a clear evidence about the function of plant Ca^{2+}-ATPases in the formation of stimulus-specific Ca^{2+} signatures (Qudeimat and Frank 2009). Ca ion release in the cytosol should later on activate different cascades of molecular pathways to orchestrate plant response. Cellular calcium also has a critical role in gene expression regulation for plant defense genes against various stresses. There is need to do advanced research to rise the limitation of information about genes response to calcium signals specifically (Behera *et al*., 2018)

At the molecular level, there are different molecular and signaling mechanisms is activated in response to extreme temperatures and drought as in activation of MAP kinases, regulatory proteins (late embryogenesis abundant proteins "LEA", molecular chaperons and heat shock proteins "HS") and activation of detoxification enzymes involved in detoxification of reactive oxygen species (ROS) (Dos Reis *et al*., 2012).

Genes responsible for the production of heat shock proteins and factors, has been identified as part of the common proteins that are overly expressed in plants under drought stress inducing conditions (Guo *et al*., 2016). Heat shock protein (HSPs) tend to work as chaperons to protect, minimize denaturation "and miss-folding" of protein, HSPs and HSFs (proteins that stimulate the expression of HSPs and other tolerance genes) considered as a molecules involve in mechanisms that plant uses to expand their tolerance for different stresses (Sun *et al*., 2002). HSFs are part of a large gene family transcription factors in plants, and found in high redundancy that control response of plant under abiotic and biotic stresses (Miller and Mittler 2006). Generally plants have greater number of HSFs genes compared to animals, fungi and bacteria (Guo *et al*., 2016). In bacteria, HSFs tend to be involved in several protective mechanism needed for survival under unfavorable environmental conditions, as these tends to work as proteases (HSPs) and molecular

chaperones (HSFs), and their level tend to increase upon exposure to a range of stresses (Roncarati and Scarlato, 2017). They tend to form homo- or heterotrimers to alter nuclear localization to enhanced or suppressed transcription of genes *via* bind to a cis-acting element (nGAAnnTCCn) at the promoter region of genes, or potentially bind and activate their own promoters, as well as promoters of other members of their own family. In general, the expression of HSPs is mainly regulated by heat shock transcription factors (HSFs) (Yang *et al*., 2016).

In the present study, we exposed sugar beet seedlings to different PEG levels (0%, 3%, 5% and 7%), and isolated RNA from these plants to clone and characterize HSFs. We further cloned the HSFs into plant expression vector and used it to transform *Arabidopsis thaliana*.

Materials and Methods

Drought stress experiments*:* Sugar beet seeds variety Farida were kindly donated from Sugar Crop Research Institute, Agriculture Research Center (ARC), Giza, Egypt, and germinated on wet cotton for one week before transferring into perlite and sand-filled pots in greenhouse. The plants were kept in the greenhouse at temperature range of 16-24°C; the plants were irrigated with $_{1/2}$ strength Hoagland solution (Hoagland and Arnon, 1950). Drought stress was applied to plants 30 days post transferred into the greenhouse. The plants were removed from the soil and were placed in plastic containers (35 × 55 × 15 cm) filled with Hoagland solution and supplemented with 0, 3, 5 or 7% (w/v) polyethylene glycol PEG 6000. The Hoagland solution, supplemented with the corresponding PEG level, were refreshed every 2 weeks (Hoagland and Arnon, 1950 & Saglam *et al*., 2014). One-month post drought exposure leaves of sugar beet plants were harvested and stored in liquid nitrogen.

RNA extraction: A weight of 200 mg plant tissues was grinded in liquid nitrogen with mortar and pestle, extraction was performed using SV Total RNA isolation kit (cat. # Z3100, Promega, USA). RNA samples were measured using NanoDrop™2000 spectrophotometer (Thermo-Fisher Sci., Germany). To eliminate DNA residues from samples, RNA samples were treated with DNase which included in the kit. RNA was visualized on 1% agarose.

cDNA synthesis: Synthesis of cDNA was carried out using RevertAid™ Premium First Strand cDNA Synthesis Kit (cat. # K1621, Thermo-Fisher Sci., Germany).

Real time-PCR: PCR reactions were prepared in a total volume of 15µL contained 7.5µL iTaq™ universal SYBR® Green Supermix (cat. # 172-5121, BioRad, USA), 2 µL of 10 pmol concentration forward and reverse primers (5'CATTGGGAGAGCGTGTGAAG3', 5'AGACTGTCGCT AGTTGAGGC3'). The rest of the volume was adjusted with nuclease-free water up to 15µL. PCR was performed using a 7500 Real-Time PCR System (Stratagene Mx3005P) in 96-well plates as follow:- 5 min at 95 °C, 40 cycles of 5 sec at 95 °C, and 1 min at 60 °C. Each PCR reaction was repeated three times, with two biological samples. To determine the correct amount of cDNA template in each sample standard curves were organized using three different dilutions of the template, following the procedure of (Larionov *et al*., 2005). Glutamine synthetize gene was used as a control in all real-time PCR experiments using the following primers F-5'GACCTCCATATTACTGAAAGGAAG3' and R-5' GAGTAATTGCTCCATCCTGTTCA 3' which amplify 110 bp (Taski *et al*., 2012). The relative quantification expression of target genes was calculated with comparative cycle threshold (Ct) method (Livak and Schmittgen 2001), according to Equation : ΔCt = (Ct target gene – Ct housekeeping gene); ΔΔCt = ΔCt treatment –ΔCt control. Relative expression ratio (R) = $2^{-\Delta\Delta Ct}$.

Statistical analysis: The results were presented as mean ± standard error (SE), and real-time PCR data were analyzed using Graphpad online software (www.graphpad.com) and by using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA).

***Cloning of full-length heat shock factor cDNA*:** One-month post osmotic-stress treatment (7 % PEG 6000), total RNA was isolated from plants using SV Total RNA isolation kit (cat. # Z3100, Promega, USA), following the manufacturer provided protocol. For RT-PCR, Super Script®lll One-Step RT-PCR kit with platinum® Taq Polymerase (Cat. # 12574018 Invitrogen™. Germany) were used. The reaction was performed in 50 µL volume consisting of 25 µL 2X reaction mix, 2 µL SuperScript™ III RT/Platinum™ Taq Mix, 1 µL template RNA, 1 µL of Sense primer with *Xba*I restriction site (10 µM) HSF 5'AATCTAGAATGGAA CCCAAAACTG3', HSF Anti-sense primer (10 µM) 5'AAGAGCTCATTTGCAAATCAAG CTGG3' with *Sac*I restriction site, 8 µL MgSo4 and 12 µL RNase free-H_2O. Tubes were incubated at 55° C for 30 minutes, followed by incubation at 94 °C for 2 min. The amplification was carried out for 40 cycles (94°C for 15 seconds, 60°C for 30 seconds then 68°C for 1 minute) followed by final extension at 68°C for 5 minutes. The PCR product was visualized on 1% agarose gel. The 1030 bp amplified fragment was cloned into p GEM®-T Easy Vector (Cat# A3600, Promega, USA), the cloning steps was as the following: 5 µL 2X Rapid Ligation Buffer, T4 DNA Ligase, 1 µL pGEM®-T Easy Vector (50ng), 1 µL T4 DNA Ligase and 2 µL of (25ng) product PCR, final volume was adjusted to 10µL using nuclease-free water, the reaction was mixed via pipetting, followed by overnight incubation at 4°C.

Transformation into E. coli: The pGEM-T easy vector carrying the full-length HSF gene was transformed into DH10β *E. coli* cells *via* heat shock. Competent cells (50 µL) along with ligated DNA were incubated on ice for 5-10 min in a tube. The mixture was then placed in a thermostatic mixer at 42° for 45 seconds before re-incubating the tube on ice for further 10 min. 1 ml of LB broth was added to the mixture in each tube, and then further incubated for 2 hr in a shaker incubator at 37°C. For white/blue screening, transformed cells were then plated on X-gal–IPTG LB plates 40 µl (100mM) IPTG and 120 µl X-Gal (20 mg/ml fortified with 50 mg/l kanamycin, and incubated overnight at 37°C. White-coloured colonies were picked and immediately inoculated in 5 ml LB broth medium containing 50 mg/ml kanamycin, and placed in shaker incubator overnight at 37°C with 200 rmp. The next day, 1.5 ml of cell culture were harvested and plasmid DNA miniprep was conducted using Qiagen Miniprep kit (Cat. # 27104).

Bioinformatics analysis: pGEM-plasmid carrying the cloned fragment was sequencing at Lab Technology©. Nucleotide sequence of isolated gene was assembled using Codon Code

Corporation software (https://www.codoncode.com/aligner/index.htm), then blasted with (http://blast.ncbi.nlm.nih.gov/Blast). Multiple sequence alignment was conducted using CLUSTALW (http: //www.genome.jp/tools-bin/clustalw) (Thompson *et al*., 1994). Phylogenetic tree was generated using ETE Toolkit (Huerta-Cepas *et al*., 2016).

Motif-based sequence analysis: Protein sequences is powerful computational tool available used to compare unknown structure and function sequence with well characterized structure and function sequence according to blastx result (Higgens and Taylor, 2000). ExPasy software (https://web.expasy.org/translate) was used for translating the sequence to amino acids (Gasteiger *et al*., 2005).

Cloning of HSF gene into plant expression vector: The plant binary vector pRI201-AN (Cat. #3264-3267, Takara Bio Inc) was used for sub-cloning the *HSF* gene. The first step was carried out *via* digesting the recombinant pGEM-*HSF* plasmid and the pRI201-AN vector with *Xba*I and *Sac*I restriction enzymes (*Cat. # FD0684* and *FD1133,* Thermo Scientific, USA) at 37°C for 30 min. This was followed by visualizing on 1.5% agarose gel. The 1030 bp fragment was excised from the gel and purified using the DNA Clean & Concentrator™ (Cat. # D4013 Zymo Research). The purified fragment was eluted with 30 µl ddH_2O, and then sub-cloned into the pRI20-AN binary vector. A ratio of 1:3 vectors: insert DNA was mixed with 1 µl T4 DNA ligase (Cat. # EL0011. Thermo Scientific, USA) and 10X ligation buffer to final volume of 10 µl. The reaction was kept at room temperature for an hour. About 100 ng of the ligated vector (pRI201-AN-HSF) was then used to transform freshly prepared *Agrobacterium tumefaciens* strain (GV3101) competent cells.

Cloning of recombinant plant expression vector into Agrobacterium*:* Transformation of BvHSFA1 construct into *Arabidopsis*: *Arabidopsis* seeds were grown in petri dishes at 4° C, then transferred to pots under plastic cover to reach the requested high humidity until shoots are well developed. When plants start to blooming the opened flower was removed and shoots were dipped in agrobacterium cells solution which contains *HSF* constructed vector to transfer the gene into the closed flowers (Tsuda *et al*., 2012). Inoculated plants were covered with dark paper bags for 2 days to allow DNA transfer to plant cells. By the end of life cycle of the plants, seeds were collected and screened for transgenic seeds.

Isolated plasmid (pRI201-AN-HSF) was mixed with the 100 µl of Agrobacterium competent cells in an eppendorf tube and was incubated on ice for 30 min. This step was followed by incubating the tubes in the thermostatic mixer at 37°C for 5 min, then further incubation on ice for 5 min. 1 ml of LB broth was added to each tube then incubated in a shaker incubator for 4 hr at 28 °C. 100 µl of cultures were spread on LB agar medium containing 50 mg/ml kanamycin and were incubated for 48 hr at 28°C. Single colony was then used for further transformation of plant tissues. Arabidopsis transformation: Arabidopsis seeds were surface sterilized in an eppendorf tube with 5% Sodium hypochlorite and a drop of Tween 20 for 30 minutes followed by rinse with distilled water 5-6 times. Sterilized seed were cultured on germination medium consisting of half strength MS medium, MS basal salt mixture fortified with 20 g/l sucrose + 0.5 g 2-(N-morpholino) ethanosulfonic acid (MES). The medium was solidified using 8.0 g Agar/ liter; pH was adjusted to 5.7. and the plates were incubated at 4 °C in the dark, three days post dark incubation, the plats were transferred into growth room for 2-3 weeks (20 ±2°C with 16/8 light/dark cycle). Germinated seedlings were transferred into 10 cm pots filled with soil mixture (1perlite:1 peat moss) and were kept in greenhouse at 20°C with 16/8 light/dark hours for 3 weeks till blooming. Agrobacterium strain (GV3101) carrying the cloned HSF gene was used to inoculate 5 ml liquid LB medium containing kanamycin, after two days of incubation at 28°C it was used to inoculate 500 ml liquid LB broth with kanamycin overnight at 28° C till reaching an O.D. of ≈1.5-2.0. The Agrobacterium cells were centrifuged at 4,000 rpm for 10 min, and harvested cells were re-suspended in freshly prepared 5% sucrose solution with one drop of Tween®20. Arabidopsis floral parts were dipped into the Agrobacterium suspension for 1-2 min with gentle shacking, and then plants were covered with plastic stretch wrap. 24one day post treatment, the plastic cover was removed. This procedure was repeated twice, with 10 days interval. Screening of Arabidopsis: Mature Arabidopsis siliques were collected using a piece of paper and sieve mesh; about one thousand seeds about 20 mg were germinated on germination medium fortified with 50 mg/l Kanamycin. The plates were incubated in growth room (20 ±2°C with 16/8 light/dark cycle) for 7-10 days. Green and healthy-looking seedlings with true leaves and roots were transferred to fresh selection medium. DNA was isolated from individual seedlings using DNeasy Plant DNA mini kit, (Cat.# 68163 QIAGEN), and PCR analysis was performed using gene specific HSF primers to confirm the transformation. PCR product was visualized on 1% agarose gel.

Results and Discussion

Several researchers reported that drought stress is a limiting factor at the initial phase of plant growth. In general, water deficit causes a reduction of shoot growth, number of shoots and leaf surfaces, in addition to changes in photosynthetic pigment content so chlorophyll gradual loss, causing leave color change. (Kusaka *et al*., 2005; Kuykendall *et al*., 2008; Jaleel *et al*., 2009; Anjum *et al*., 2011). Drought stress also negatively affect fresh and dry weight of different plant genotypes, which has been reported by Moosavi *et al*. (2017). On the other hand, the sugar beet roots found to be moderately tolerant to drought (Brown *et al*., 1987). Thus, the expression of heat shock factor BvHSF, which play a role in during drought stress in sugar beet was isolated and cloned.

Drought stress experiment

Polyethylene glycol (PEG) with different strength has usually been used for inducing drought stress. Due to molecular weight of PEG, it cannot pass through the cell wall and therefore it is used to regulate water potential and osmotic effect induction (Türkan *et al*., 2005; Saglam *et al*., 2014). In this study, three concentration of PEG (3, 5 and 7%) have been used to induce drought stress in sugar beet seedlings, the growth parameters, root length; leave fresh and dry weights were recorded compared with control. The difference of sugar beet growth under different PEG treatments was observed (Fig. 1.A). Root length, shoot fresh and dry weight under PEG conditions has been recorded. Results showed significant morphological changes in PEG treated sugar beet seedlings.

Interestingly, it was observed that with the increase in PEG concentration, roots length increased from 19.8 to 27.6, 29.8 and 31.2 at 3, 5 and 7% PEG, respectively (Fig.1.B). It is known that water stress in culture can adversely affect plantlet growth, with various differences in genotypes response (Gopal and Iwama, 2007). Our results clearly showed a negative reduction on fresh and dry weight (due to PEG treatment), with a 40 g and 5 g reduction in fresh and dry weight respectively at 0 and 7% PEG, (Fig. 1. C and D). The obtained results were in consistent with (Hajheidari *et al*., 2005 & Farooq *et al*., 2012); they reported that water deficit caused decrease in leaf growth and development, shedding of aged leaves as a way to coup-up with water deficit conditions, sensitivity of cells division, and decrease in nutrient uptake.

Drought stress in sugar beet was studied using real time qPCR. The cDNA was synthesized from RNA templates extracted from the different sugar beet seedling grown on PEG with concentrations. The cDNA samples ranged between 100 ng/µl to 160 ng/µl were used as a template of specific PCR as assessed for BvHSF gene using primer pairs amplify a 227 bp fragment of HSF gene. Studying the regulation of heat shock factor *BvHSF via* RT-PCR proved to be an efficient and accurate method, as it analysis the expression of other candidate genes involved in sugar beet reaction to water stress (Bechtold *et al*., 2013). Result showed significantly differences in the expression of BvHSF gene obtained from different cDNA templates. Drought treatment cause a significant increase in BvHSF transcript fold increase (4.1, 10.4, and 11.5 folds increase at 3, 5, and 7% PEG, respectively), (Fig. 2). It was clear that *BvHSF* gene was up regulated under drought stress in sugar beet, which is in line with previous workers that indicated that BvHSF belongs to a gene family involved in abiotic stresses (Swindell *et al*., 2007; Al-Whaibi 2011 & Xu *et al*., 2012).

Isolation and Cloning of the BvHSF from sugar beet: The heat stress transcription factor HSF (1030bp) was isolated using RT-PCR. The full-length fragment BvHSF was successfully amplified from the extracted RNA from sugar beet grown on PEG (Fig. 3. A), cloned into the PCR cloning vector pGEM-Teasy, then, transferred into DH10β cells. The white colonies of the transformed *E. coli* were screened for positive clones by releasing the inserted fragment with *Eco*RI digestion. The full-length BvHSF DNA was sequenced and assembled nucleotide sequences was submitted to GenBank (Accession no. MG58535).

Bioinformatics analysis

Multiple sequence alignment: The obtained sequence of *BvHSF* gene isolated from sugar beet was aligned against similar sequences in the GenBank using Basic Local Alignment Search Tool for Nucleotides (BLSTN). When analysing amino acid sequence of the cloned *BvHSF* gene vs. available *HSF* genes sequences presented in database (summarized in Figure 4A), 4 major clades (designated as A, B, C, and D) were performed. Clade A is clearly the most distant one from the rest of the clades (represented by *Ziziphus jujube*); clade B is represented by members from *Fabaceae* family and *Gossypium arboreum*; clade C is represented by large number of individuals. Clade D is mainly composed of different clones from *B. vulgaris* along with closely related individuals as in *Chenopodium quinoa* and *Spinacia oleracea*. Differences among clade D are very minor with almost 99% identity.

Interestingly, further analysis at amino acids level between isolated *BvHSF* and *B. vulgaris* susp. Vulgaris heat stress transcription factor A-7a (XM_010698225.2), showed three point mutations; 1) asparagine replaced by serine at position number 121 (N121S), 2) change of serine to glycine in position 233 (S233G); 3) change the threonine to alanine in position 269 (T269A). Serine, glycine and alanine are small, non-polar amino acids that can work as a start for side chains, therefore structural substitution of a small side chain for a large one can be disastrous in some proteins (Barnes and Gray, 2003).

In addition, further motif-based sequence analysis was performed for *BvHSF* vs. other similar proteins motifs. Figure 4B clearly shows similarities between *BvHSF* and other heat shock factors from other species. The *BvHSF* protein was found to have the following motifs: DNA binding domain (DBD) and oligomerization domain (OD), nuclear localization signal (NLS), nuclear export signal (NES) and activator motifs (AHA motifs) which represent in the figure with different colors (Liao *et al*., 2016). Our results indicate that the *BvHSF* protein belongs to class A Hsfs as it contains of AHA motif, which are essential for transactivation. Unlike class A Hsfs, class B and C Hsfs lack AHA motifs. Class B Hsfs contain a repression domain instead of an AHA motif. HSF class plays role as the main regulators of heat shock response and take part as imperative components in abiotic stress (Liu *et al*., 2011)

Introduce BvHSFA gene into Arabidopsis:

Subcloning of the Bv*HSF*A into plant binary vector: Both BvHSFA DNA and pRI201-AN plasmid DNA were individually digested with *Xba1 and Sac1* to release the BvHSFA fragment, and linearized the plasmid, respectively. The released fragment and linearized vector were purified from agarose and ligated together, then transformed to *E. coli* cells. The successfully ligated *BvHSFA* were screened by digestion with *Xba and Sac* and colony PCR using full-length and partial (core) target primer (qHSF) for heat shock factor (Fig. 3). The recombinant pRI201-AN-BvHSF DNA was then transferred into Agrobacterium GV3101 cells, subsequently; floral dip transformation with GV3101 containing pRI201-AN-BvHSF was applied.

Screening and Molecular analysis of transgenic plants.

Transgenic T0 Arabidopsis seeds grew on kanamycin MS medium. The putative transgenic seeds were survived and grew with good performance were counted, applied to molecular analysis to confirm the transgenes. The extracted DNA samples were subjected to PCR analysis using core HSF primers (qHSF). The positive transgenic seedlings were able to amplify a fragment with a size of 227 bp. Only 5 plants out of 1000 seeds were survived on kanamycin and produced seeds after transferring to the greenhouse, representing 0.5% transformation frequency (Fig. 5). This ratio was in constant with Clough and Bent (1998) and Bent (2006), who reported that transformation ratio of floral dip transformation in Arabidopsis ranged between 0.1- 3%.

Conclusion

In this investigation BvHSF gene from sugar beet has been studied using qRT-PCR after applying different concentration of PEG. Results showed that BvHSF was up-

regulated in high concentration of PEG (5 and 7%). Thereafter, BVHSF gene was isolated, cloned in pGEM-T Easy, sub-cloned into PR vector to be used in floral dipping.

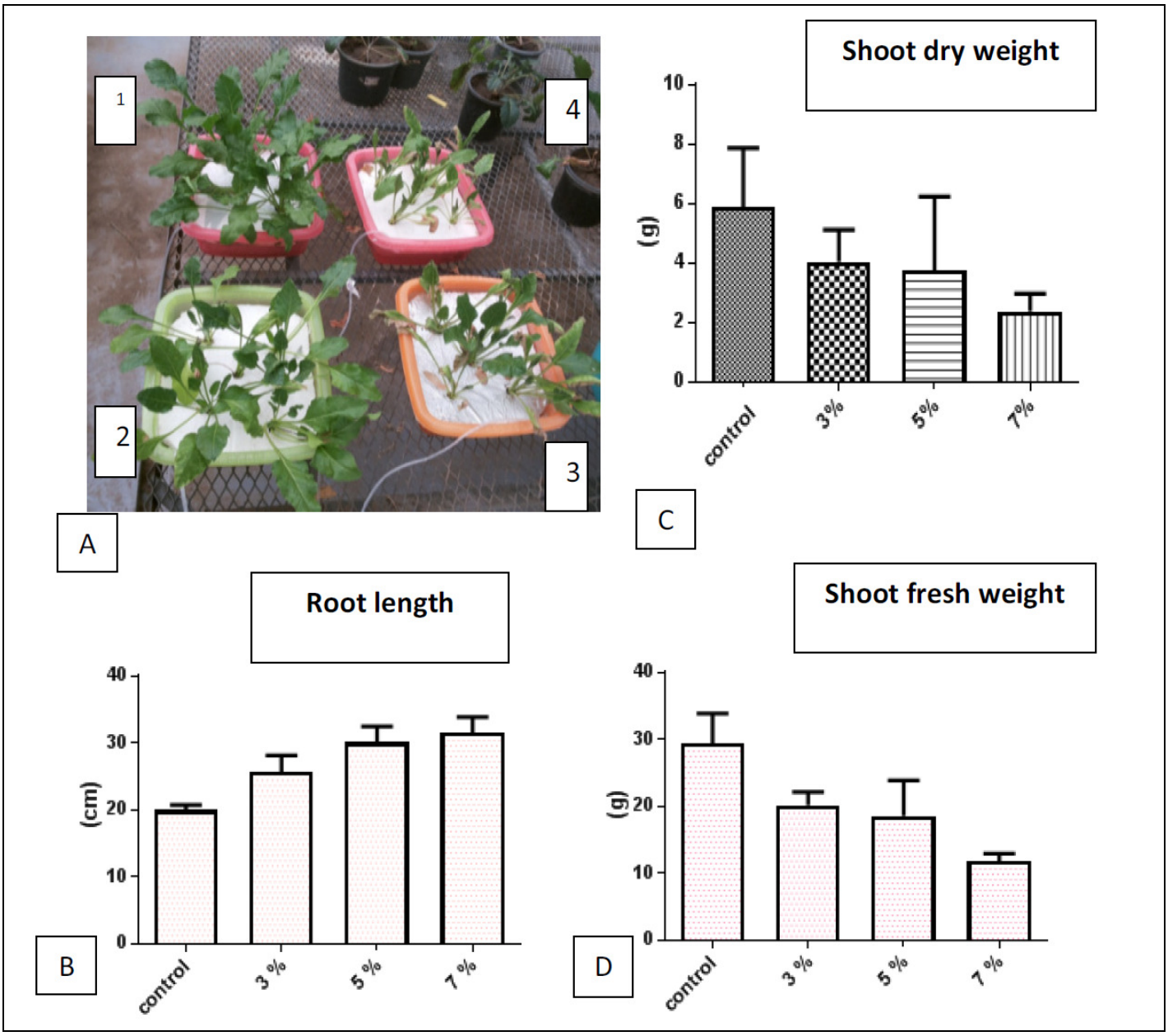

Fig. 1 : (A) Morphological characterization of greenhouse PEG-treatment of sugar beet plants in closed-hydroponic system (1: 0%, 2: 3%, 3: 5% and 4: 7% PEG)
The effect of different concentration of polyethylene glycol (PEG 6000) 0 %, 3 %, 5 % and 7 % on root length (B), fresh and dry, shoot weight (C&D).

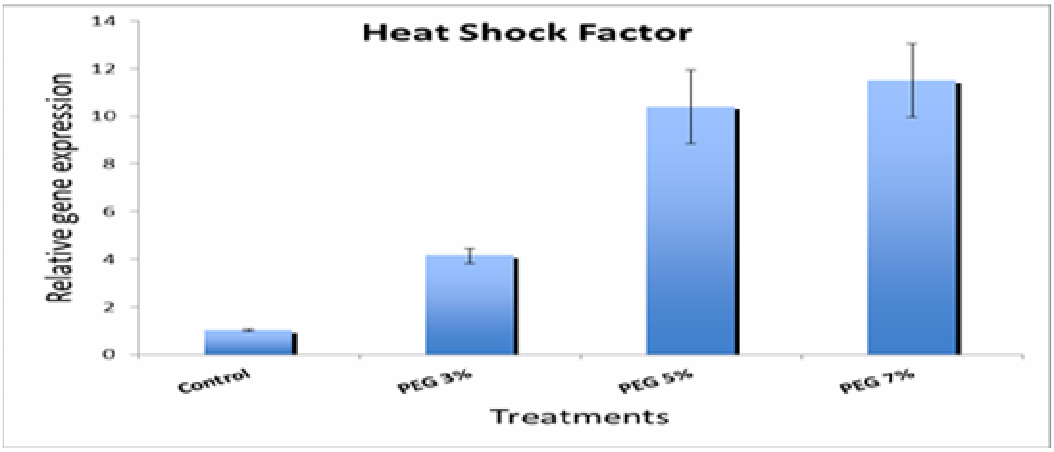

Fig. 2 : Relative gene expression for BvHSF in sugar beet control (1) and PEG treatments (2,3 and 4)

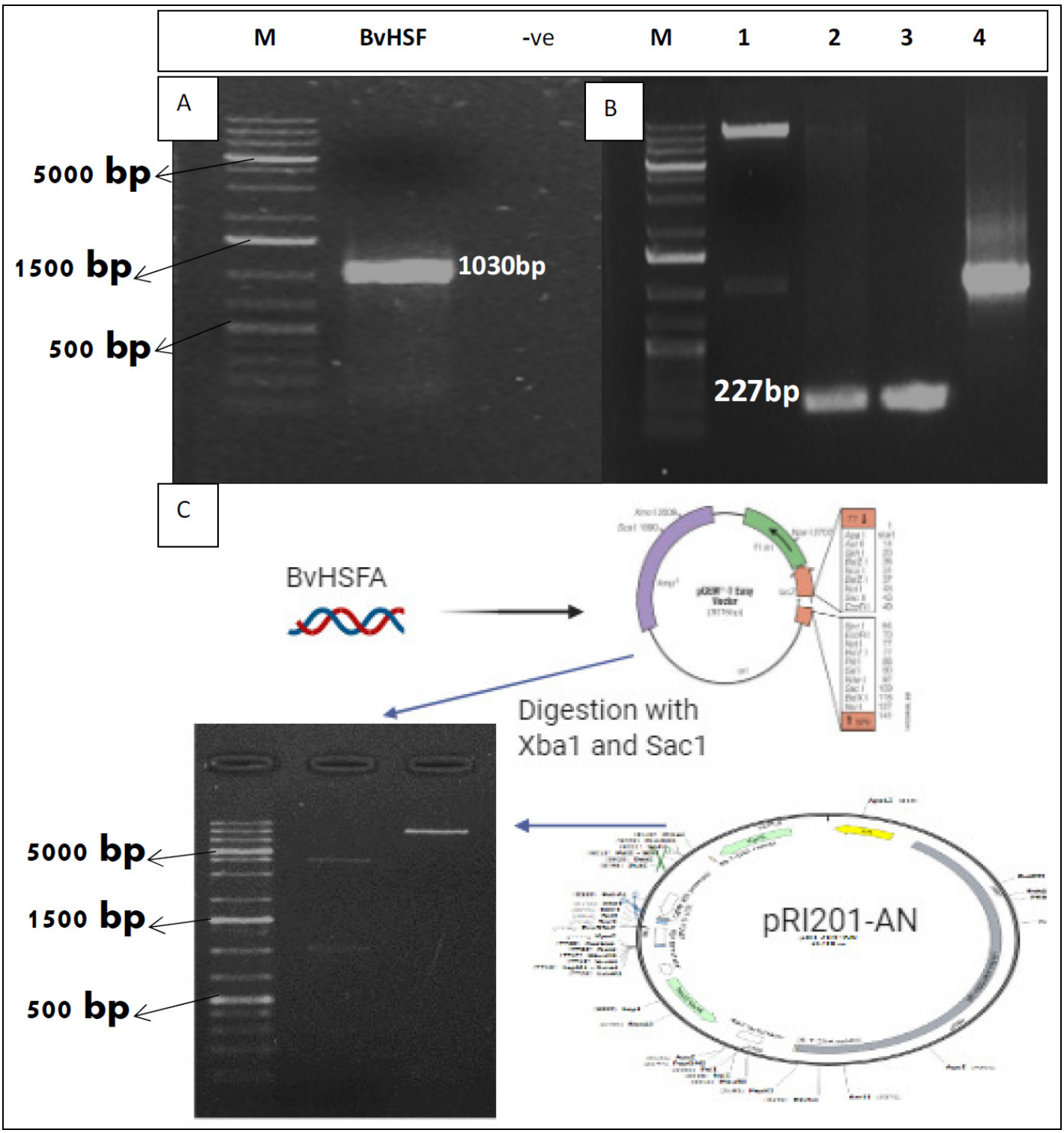

Fig. 3 : Cloning of the BvHSf gene from sugar beet into plant expression vector.
(A) Gel electrophoresis of amplified full-length cDNA of HSF gene from sugar beet (1030 bp) M : 1Kb plus.
(B) Agarose gel electrophoresis showing: confirmation by Double restriction digest lane 1 , amplifying of 227 bp fragment using primers were used for qPCR lane 2&3, also full length primer used with DNA temple from sugar beet as a positive control lane 4.
(C) Diagram of steps of BvHSF by digestion both pGEM-plasmid carrying the cloned and pRI201-AN vector with xbaI and SacI restriction enzymes to sub-clone BvHSf into pRI201-AN vector.

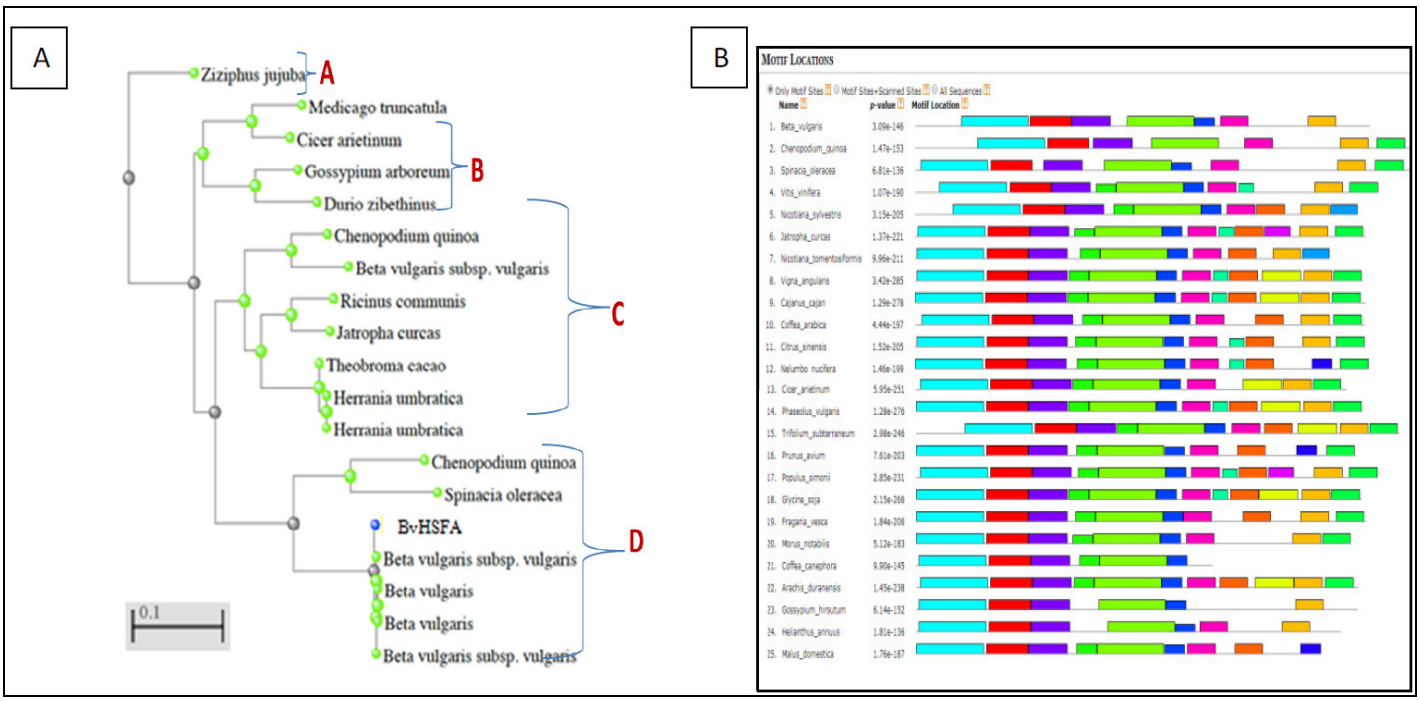

Fig. 4. (A) Phylogenetic analysis of isolated heat shock factor.
(B) MEME Motif-based sequence alignment result based protein sequence of HSF against Proteins.
Motif 1: DNA binding domain (DBD), Motif 2: Oligomerization domain (OD),
Motif 3: Nuclear localization signal (NLS), Motif 4: Nuclear export signal (NES),
Motif 5: activator motifs (AHA motifs).

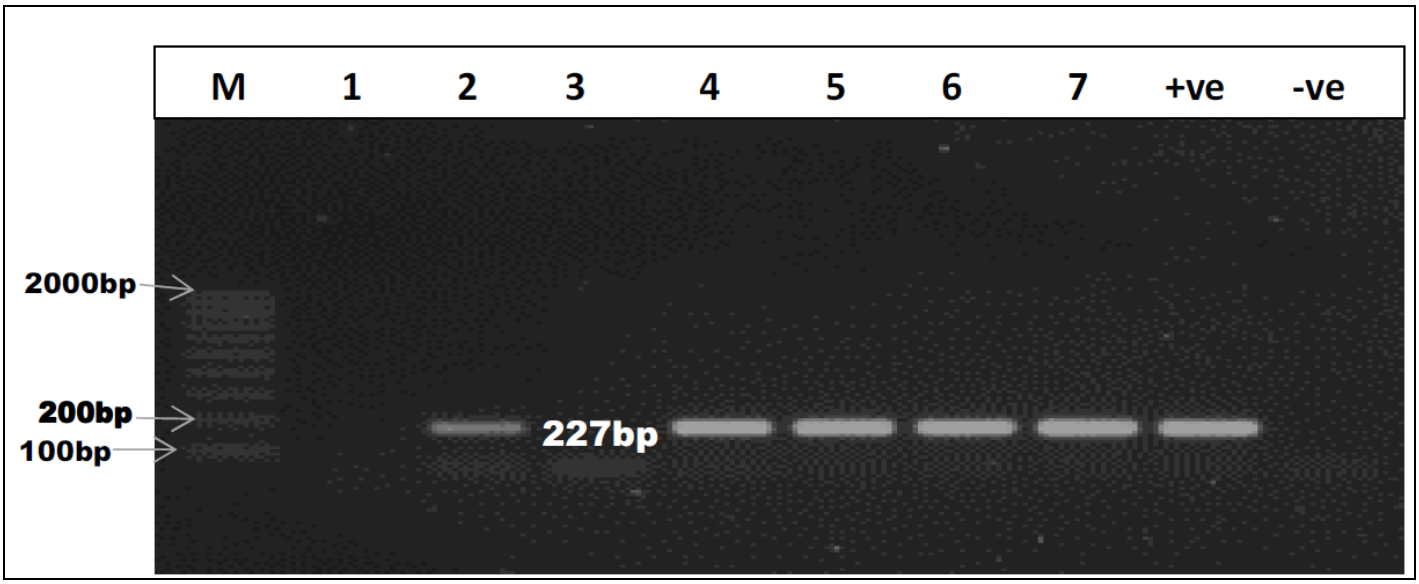

Fig. 5 : PCR analysis using core primers of hsf gene for T0 Arabidopsis. M: 100 bp ladder DNA marker; +ve and -ve : represents positive and negative control; lanes 2, 4, 5, 6 and 7 showed positive amplified fragment of 227 bp, lines 1 and 3 are non-transgenic.

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