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Full Length Article

Overexpression of *Nicotiana tabacum HSP17.6* Enhances Abiotic Stress Tolerance in *Brassica napus*

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Abstract

Small heat shock proteins (sHSPs) are ubiquitous and play critical roles in plant development by protect them against biotic and abiotic stresses. However, the reports suggesting the role of sHSPs in *Brassica napus* against stress resistance are surprisingly rare. Here we isolated sHSP17.6 from *Nicotiana tabacum* and explore its function in *B. napus* under different abiotic stresses. Phylogenetic analysis and subcellular localization prediction revealed that *NtHSP17.6* is a cytosolic class I protein. *B. napus* plants overexpressing *NtHSP17.6* exhibited high expression level, water content, proline, soluble sugar and chlorophyll content than wild type under heat stress. Similarly, normal root growth and more leaves biomass were observed in *B. napus* overexpressing *NtHSP17.6* under salt and drought stresses. These overexpression analyses suggested that *NtHSP17.6* in *B. napus* confers high resistance against heat, drought and slightly towards salt stress. These findings will be helpful to develop economic stress tolerant crops and avert yield loss. © 2020 Friends Science Publishers

Keywords: Abiotic stresses; *Brassica napus*; *Nicotiana tabacum*; sHSP17.6; Overexpression

Introduction

During growth and development, plants are usually exposed to a broad range of abiotic stresses (Rana *et al*. 2004), such as high temperature, salinity, drought, and cold during their growth and development. Such abiotic stresses significantly affect the growth of the plant species and result in reduced crop yield (Ahuja *et al.* 2010). To encounter these stresses, plants developed complex strategies and adapt themselves to the extreme environmental conditions to ensure their survival (Hellmann and Estelle 2002). As, many genes are rapidly induced in plants due to combat these stress conditions, one of which is HSPs (Ahuja *et al.* 2010; Ruibal *et al.* 2013). HSPs serve as molecular chaperones to inhibit protein accumulation and thus regulate the homoeostasis of protein folding to endure heat stress in plants (Haslbeck and Vierling 2015). Based on their molecular weight and sequence homology, plant HSPs are categorized into five separate groups: HSP100s, HSP90s, HSP70s, HSP60s and HSP20s or small heat shock protein (Kotak *et al.* 2007).

In contrast to other HSPs, small heat shock proteins (sHSPs) exhibits two distinctive features as they bind to substrate protein without using ATP and also possessed tremendous ability to bind with denatured proteins (Waters 2012). The sHPSs form 200–800 kDa mulimetric chaperon complexes (Lee *et al.* 2012) and based on sequence similarity, they are categorized into six classes (Siddique *et al.* 2008), such as Cytosolic Class I, II, and III localized to the cytosol or nucleus (Li *et al.* 2016a), as well as the endoplasmic reticulum (Liu and Howell 2010), mitochondria and plastids (Waters *et al.* 1996). Though plant sHPSs were primarily identified in response to the heat stress, while later it was revealed that sHPSs also induced in response to other abiotic stresses such as cold (de Azevedo Neto *et al.* 2006), oxidative stress (Yang *et al.* 2006) and drought stress (Jiang *et al.* 2009). In addition, sHSPs were also produced particularly in the reproductive organs at definite developmental stages such as maturation and germination, development of pollen and fruit maturation (Yang *et al.* 2012). For the first time, Sun *et al*. (2001) explored the role of *AtHSP17.6* under stress conditions and reported that *AtHSP17.6* induced during seed development stage along heat stress, but not with salt stress. They further confirmed that overexpression of *AtHSP17.6* could induce tolerance in plants against heat and salt stress. Similarly, the role of HSP17.6 was reported in *N. tabacum* against heat stress (Yoon *et al.* 2005). Later, Koo *et al*. (2015) reported that *NtHSP17.6* also enhanced seed germination rate under light dependence and heat shock.

Rapeseed (*Brassica napus* L.) is an important oilseed crop globally, offering high quality of oil with low saturated fats. However, adverse abiotic stresses such as heat, cold, salinity, and drought induce a negative impact on the yield and quality of rapeseed. Drought stress causes dreadful effect

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on vegetative and reproductive phases of oilseed rape (Zarei *et al.* 2010), whereas salt stress reduces plant growth and production by inducing osmotic stress, which closes the stomata and ultimately reduces the photosynthesis activity (Rezaei 2017). However, the sensitivity of rapeseed against abiotic stresses, particularly towards heat stress is reported in different studies (Gan *et al.* 2004; Young *et al.* 2004). Further, Kutcher *et al.* (2010) reported the sensitivity of rapeseed against heat stress, demonstrating that yield of rapeseed reduced to 75 $kgha^{-1}$ by every increase in 1°C temperature, whereas, as the temperature surpass to 30°C during growing season, the yield significantly reduced to 180.4 $kgha^{-1}$ per day. High temperature during seed filling phase constantly interrupt normal seed development, which enhances the probability of desiccated, abnormal and lower quality seeds (Peltonen-sainio *et al.* 2011). As the global earth temperature is rising every year, it is expected that by 2050, the temperature may be increased by around 3^{o}C (Gornall *et al.* 2010) and thus maximizing the crop production in this rapidly changing climate will be a formidable challenge (Hampton *et al.* 2013).

To investigate the plant stresses, it is quite necessary to cautiously measure the timing, duration and intensity of stress (Driedonks *et al.* 2016). Therefore, genetic engineering of economical crops are the most feasible and convenient approach to avert yield loss and produce stress resistant crops capable of efficient adjustment in a particular local environment and varying climate conditions. Due to the role of HSP17.6 under heat stress particularly at late stage of seed development and ability of *N. tabacum* to tolerate broad range of climate change (PIER 2014), altogether make it quite worthy to explore the function of *NtHSP17.6* and further, can be used to develop stress tolerant plants. Although past few years have witnessed considerable research to understand the function of sHSPs, but their biological role under different stresses still need to be thoroughly examined. Strategies adopted by plants to survive in extreme environments include physiological regulation, morphological changes and behavioral adjustment. The role of sHPSs has been well proven in many organisms ranging from fungi to plants in order to protect the cell in case of damage under different abiotic stresses. Despite of considerable research on the role of sHPSs under stress environment, few studies reported sHPSs in *B. napus*. Therefore, here, we reported the isolation of *sHSP17.6* from *N. tabacum* and assessed its function by developing *B. napus* overexpressing *NtHSP17.6*. Transgenic *B. napus* exhibited resistance against heat, drought, and salt stresses that actually seems to be the desirable gene to confer resistance in *B. napus* that will be further explore in detail for several aspects.

Materials and Methods

Plant materials and growth conditions

Tobacco (*N. tabacum*) plant was used to isolate heat shock protein (HSP17.6) and to assess its expression in *B. napus* under various treatments. *N. tabacum* was grown under following conditions: 18–22°C with 200 μmol light intensity, 60–70% relative humidity along 16 h light/8 h dark photoperiod cycle. Leaves were collected for RNA extraction and stored at -80°C.

RNA extraction and cDNA synthesis

According to the manufacturer's instructions, total RNA was extracted from the leaves samples using Trizol method (Invitrogen, Carlsbad, CA). RNA samples were treated with RNase-free DNase I (Takara) to avoid DNA contamination. Concentration of RNA was assessed by a NanoDrop ND-1000 spectrophotometer. cDNA was synthesized from 3 µg RNA using a PrimeScript Reverse Transcriptase kit (Takara, Shiga, Japan).

Subcellular localization of *NtHSP17.6*

Coding region of *NtHSP17.6* excluding the stop codon was amplified by PCR using given primer HSP17.6F/R in (Table 1) and cloned into pENTR/D-TOPO vector and positive colonies were selected through PCR verification and sequenced by Invitrogen (Sangon, Shanghai). An expression vector was constructed by performing LR recombination reaction between pENTR/D-TOPO entry clone to destination vector PK7FGW2.0. Then, PK7FGW2.0-*NtHSP17.6*-eGFP and PCX-IND-DsRed and P19 was transiently transferred to the leaves of *N. benthamiana* using *Agrobacterium* strain GV3101. However, the mixture of PK7FGW2.0.-eGFP and PCX-IND-DsRed and P19 was used as a control. Three days after transformation, expression of the *NtHSP17.6*-eGFP fusion protein with control was examined using confocal microscopy (Leica TCS SP5 II system, Leica, Wetzlar, Germany).

Plasmid construction, sequence analysis and plant transformation

Polymerase chain reaction (PCR) was performed to amplify the full-length open reading frame (ORF) of *NtHSP17.6* using synthesized *NtHSP17.6*-F/R primers mentioned in (Table 1) under following conditions: 94°C for 3 min, 35 cycles of 95°C for 10 sec, 57°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 10 min. The predicted PCR product was purified and ligated into pMD18-T vector (Takara, Dalian, China) (Invitrogen) and independently transformed colonies were selected and confirmed the presence of gene through PCR and sequenced by Invitrogen (Sangon, Shanghai, China) for analysis.

BLASTP program (http://www.ncbi.nlm.nih.gov/BLAST) was used to obtain the homology of *NtHSP17.6*. The molecular weight (MW) and isoelectric point (PI) were predicted

through ProtParam tool using ExPASy database (https://www.expasy.org/ProtParam/), protein hydrophobicity (Protscale, https://web.expasy.org/protscale/) signal prediction (http://www.cbs.dtu.dk/services/SignalP/), transmembrane motif prediction (http://www.cbs.dtu.dk/services/TMHMM/) and localization was predicted through WoLF PSORT (https://www.genscript.com/tools/wolf-psort/) tool. Amino acids sequence alignments were analyzed by ClustalW program using default parameter. Neighbor joining algorithm was used to construct phylogenetic tree through MEGA 5.0 (Tamura *et al.* 2011) and Bootstrap analysis was performed with 1,000 replicates to assess the significance of nodes.

The *NtHSP17.6* coding region was inserted into *Bam-HI* and *KpnI* restriction site of overexpression vector pCAMBIA 1300 under a highly strong constitutive prompter the Cauliflower mosaic virus (CaMV) 35S, producing higher gene expression level in dicot plants. pCAMBIA 1300: *NtHSP17.6* construct was transformed into wild type (WT) of *B. napus* through floral dip method by *A. tumefaciens* strain GV3101 (Clough and Bent 1998). Transgenic plants were selected through screening of hygromycin resistance and further PCR confirmation.

Experimental treatment and qRT-PCR analysis

We induced different stresses to study the expression pattern of *NtHSP17.6* under stress conditions such as at 40°C, cold stress (4°C), salt stress (200 m*M* NaCl) and drought stress 200 g/L polyethylene glycol (PEG) in Hoagland solution. The samples were collected at 0, 2, 4, 8, 12, 24 h after treatment. Total RNA was extracted from the leaves and synthesized cDNA using PrimeScript Reverse Transcriptase kit (Takara, Shiga, Japan). Gene specific primers were designed by NCBI primer-BLAST (Table 1).

The qRT-PCR was performed using SYBR Green supermix (Takara, Dalian, China) according to the manufacturer's instruction. *B. napus* Actin gene was used as a reference gene. Relative expression level was analyzed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

After 24 h of heat stress, free proline content was assessed according to the protocol (Bates *et al.* 1973), The soluble sugars were extracted from the frozen leaves in 90% ethanol and further quantified using phenol and sulfuric acid assay with glucose as a standard (Dey and Harborne 1990), and the water loss rate was also determined. The total chlorophyll content was measured by UV spectrophotometer described by (Yang *et al.* 2009) in *NtHSP17.6* overexpressing (OE3, OE4) lines. The WT of *B. napus* "Zhongshuang 11" was used in this study.

Statistical analysis

Student *t*-test was performed to determine the significant differences between the control and stress treatments at the $p \leq 0.05$ and $p \leq 0.01$ as significance cut-offs. All the experiments were performed with three independent biological repeats and the results are expressed as the mean ± standard deviation (SD) on means.

Table 1: Sequence of primers used in this study

Primers	Sequence
NtHSP17.6-F	AAGGTACCATGTCACTGATTCCAAGAATGTTC
NtHSP17.6-R	AAGGATCCTTAACCAGAGATATCAATGGATTTG
HSP17.6-F	CACCATGTCACTGATTCCAAGAATGTTC
HSP17.6-R	ACCAGAGATATCAATGGATTTGACATCAGG
qNt17-F	TCAGACTTCCGGAGAATGCG
qNt17-R	TGACATCAGGCTTCTTCACCT
BnActin-F	TGTTGCTATCCAGGCTGTTCTTTC
BnActin-R	GATAGCGTGAGGAAGAGCATAACC

Results

Cloning and molecular characterization of *NtHSP17.6*

A full-length cDNA comprising 462 bp ORF was amplified by PCR. The gene encoded 153 amino acid protein with a MW of 17.6 KDa. The theoretical PI was 5.55, suggesting that it is an acidic protein. The results from the Protscale analysis revealed that majority of the amino acids were hydrophilic. Thus, *NtHSP17.6* considered as a hydrophilic protein. SignalP and THMM predicted that *NtHSP17.6* neither possess signal peptide nor transmembrane, respectively. In addition, Subcellular localization prediction tool WoLF PSORT indicated that *NtHSP17.6* is a cytoplasmic protein.

The multiple sequence alignment of *NtHSP17.6* with other related plant sHSPs in (Fig. 1), showing the conserved C-terminal (CS domain) of approximately 89 amino acids, which contained two conserved stress resistance regions. Phylogenetic tree was constructed based on deduced amino acid sequence of *NtHSP17.6* and sHSPs of other plant species and their subcellular localization as well (Fig. 2). *NtHSP17.6* exhibited high protein sequence similarity with *NsHSP17.6* (96.08%) *StHSP17.7* (91.56%), *CaHSP17.7* (90.26%), *MtHSP18.1* (82.39%) and *GmHSP17.5* (81.82%) and other cytosolic class II sHSPs also shared 60–70% sequence similarity with *NtHSP17.6*.

The result of *NtHSP17.6* subcellular localization indicated the localization of *NtHSP17.6* in the cytoplasm. GFP tagged *NtHSP17.6* protein was expressed under the control of strong promoter (CaMV 35S), and found that GFP dispersed throughout the cell, while the *NtHSP17.6* expression was accumulated mostly in cytoplasm (Fig. 3).

Expression analysis of *NtHSP17.6* under various stresses

Overexpression vector, which contained *NtHSP17.6* was transformed into *B. napus* plants through floral dip method. Transgenic plants were acquired by screening for hygromycin resistance and PCR confirmation. Firstly, the *cis*-elements of *NtHSP17.6* were explored to analyze the stress response of *NtHSP17.6*. The 1.5 upstream

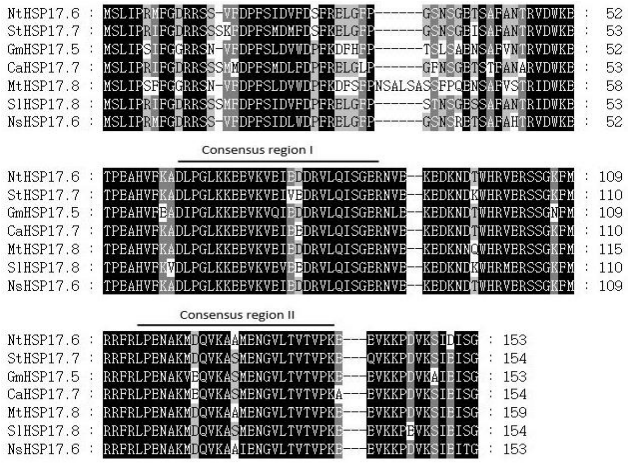

Fig 1: Alignment of deduced amino acids sequence from *Nicotiana tabacum* (*NtHSP17.6*, XP016444828.1), *Solanum tuberosum* (*StHSP17.7*, XP006350804.1), *Glycine max* (*GmHSP17.5*, XP003529343.1), *Capsicum annuum* (*CaHSP17.7*, XP016577732.1), *Medicago truncatula* (*MtHSP17.8*, XP003608277.1). *S. lycopersicum* (*SlHSP17.8*, NP001266045.1), *N. sylvestris* (*NsHSP17.6*, XP009762901.1). Identical sequences are shown in black, while conserved sequence are presented in grey color and conserved residues are underlined

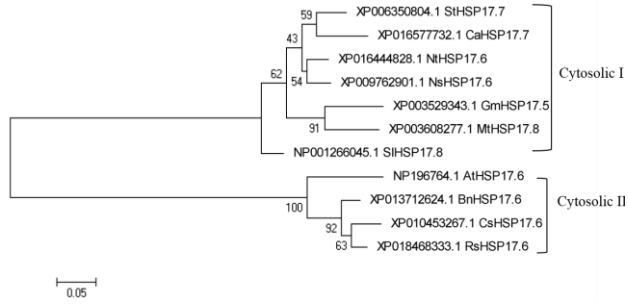

Fig. 2: Phylogenetic analysis of *NtHSP17.6* with sHSPs of other plant species. Species included in phylogenetic tree are *Nicotiana tabacum, Solanum tuberosum, Glycine max, Capsicum annuum, Medicago truncatula, Solanum lycopersicum, Nicotiana sylvestris, Arabidopsis thaliana, Brassica napus, Camelina sativa, Raphanus sativus*

to 0.5 downstream sequence from transcription start site of *NtHSP17.6* was analyzed by using PlantCARE database. Noticeably, two stress responsive elements presented in the promoter of *NtHSP17.6* and positioned near to the transcription start site, which indicated that *NtHSP17.6* could activate owing to different stresses.

The mRNA expression of *NtHSP17.6* under different stress conditions are shown in (Fig. 4). The expression of *NtHSP17.6* in *B. napus* was analyzed under heat stress. Four week old seedlings of *B. napus* were exposed under 42°C for 24 h in a controlled growth chamber. During heat stress, we consistently watered the transgenic seedlings to avoid drought stress and leaves were collected after 0, 2, 4, 6, 8, 12, 24 h. At 2 h, the expression of *NtHSP17.6* was around

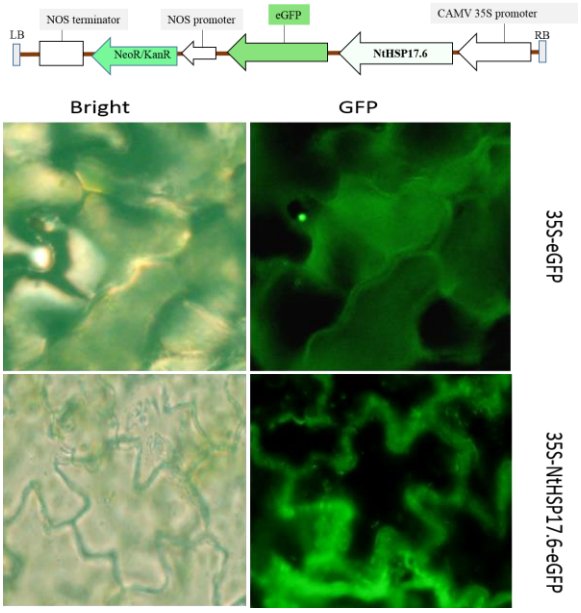

Fig. 3: Subcellular localization of the *NtHSP17.6-GFP* fusion protein in epidermal cells of *N. benthamiana.* 35S-eGFP and 35S-*NtHSP17.6*-eGFP construct was transiently expressed in the leaves of *N. benthamiana.* Epidermal cells were monitored by confocal fluorescence microscopy. Bars = 1mm

180 folds higher than the control. However, post rapid increase at 2 h, the expression of *NtHSP17.6* decreased consistently as time intervals increased (Fig. 4A).

The mRNA expression of *NtHSP17.6* under cold stress was analyzed. As, there was no significant difference at first 2 h of cold stress, at 4 h the expression of *NtHSP17.6* was increased and almost two folds higher than the former hours. However, the mRNA expression level was declined later but remained consistent later (Fig. 4B).

Four week old seedlings were treated with 200 m*M* NaCl. Then qRT-PCR performed to study the mRNA expression of *NtHSP17.6* under salt stress to investigate tolerance of transgenic *B. napus* against salinity. The consistency of mRNA expression was exhibited at first 4 h, whereas, at 8 h the expression of *NtHSP17.6* was approximately 5 times higher in stressed transgenic plants as compared to control. This pattern increased until 12 h and gradually declined afterwards (Fig. 4C).

The mRNA expression level of *NtHSP17.6* under drought stress 200g/L (PEG 4000) at different time intervals was analyzed (Fig. 4D). From 0 to 4 h, there was no notable difference in the expression of *NtHSP17.6* but at 8 h the mRNA expression level gradually started increasing and later, the mRNA expression level was 10 folds higher in stressed transgenic plants than in those of control plants at 24 h.

By employing abiotic stresses, which majorly cause detrimental effect on *B. napus*, we have found that overexpressing *NtHSP17.6 B. napus* exhibited significant tolerance against heat and drought stress as compared to salt

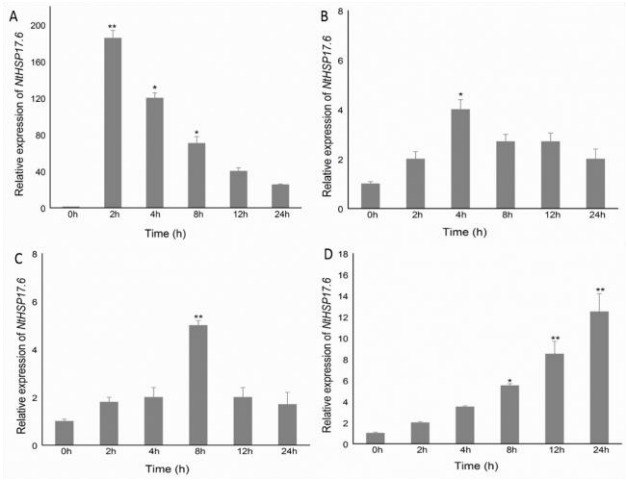

Fig. 4: The expression analysis of *NtHSP17.6 in* transgenic *B. napus* through qRT-PCR under abiotic stresses. A. Heat stress (42°C), B. Cold stress (4°C). C. Salt stress (200 mM NaCl). D. Drought stress 200g/L (PEG 4000) treatment at different time intervals. Vertical bars represents the mean ± SD from three independent biological experiments. The asterisks on the top of the columns indicate significant differences at (**P < 0.01; *P < 0.05) according to student t-test

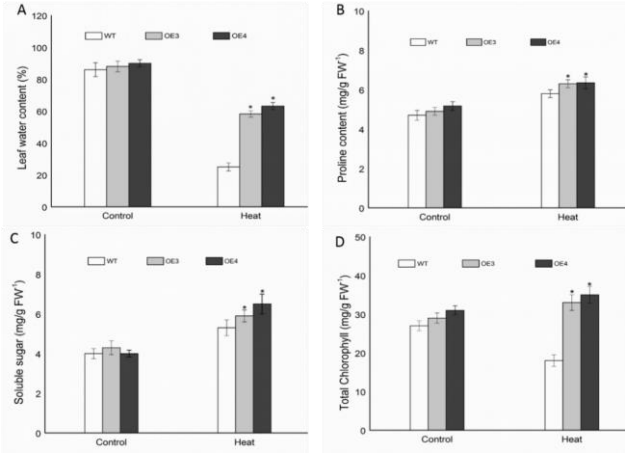

Fig. 5: Physiological characterization of *NtHSP17.6* transformed *B. napus* and WT plants. (A) Measurement of leaf water content. (B) Proline content. (C) Soluble sugar content. (D) Total chlorophyll content. Data are presented as mean ± SD from three independent experiments. The asterisks on the top of the columns indicate significant differences at (*P < 0.05; **P < 0.01)

and cold stress. However, cold stress resistance level was least significant than other stresses. Therefore, we further evaluated the effect of heat, drought and salt stress in detail.

Overexpression of *NtHSP17.6* in *B. napus* confers heat resistance

We further investigated the effect of heat in detail by measuring the physiological characteristics of transgenic and WT plants. In this study, two independent transgenic lines (OE3, OE4) were selected on the basis of higher abundance of *NtHSP17.6*, further used for different abiotic stress analysis for functional study. Post heat stress, the loss of leaf water content was comparatively less in transgenic plants as compared to WT (Fig. 5A). Additionally, under normal conditions, free proline and soluble sugar content was quite consistent between transgenic lines and WT. However, under heat stress, the concentration of free proline and soluble sugar contents were increased in transgenic plants at a quite higher level than in WT (Fig. 5B, C) and the same increment level in total chlorophyll content was also observed in transgenic *B. napus* plants (Fig. 5D).

Overexpression of *NtHSP17.6* in *B. napus* under salt stress

To further explore the function of *NtHSP17.6* under salt stress, the seeds of transgenic *B. napus* and WT were germinated on ½ MS medium containing 200 m*M* NaCl for 7 days and the lengths of their roots were measured. The root length of WT was declined by salt stress, while the root growth of transgenic seedlings was comparatively less affected due to the overexpression of *NTHSP17.6*. Under normal conditions, the root length of transgenic plants was 1.89 and 1.95 cm respectively, which were consistent with WT root length. Under salt stress, the root length of transgenic plants was 1.45 and 1.50 cm, which was 0.45 and 0.50 cm longer than the WT plant line (Fig. 6A). Under control conditions, the *NtHSP17.6* overexpressing lines did not exhibit significant difference with WT in proline content. Importantly, no noticeable difference was observed in transgenic lines and WT after salt stress (Fig. 6B).

Overexpression of *NtHSP17.6* in *B. napus* confers drought resistance

To investigate the response of drought stress in detail, the water loss of 4 weeks old seedlings withheld for 7 days followed by re-watering for 3 days, was estimated. Leaves of the same size and position on transgenic seedlings and WT were trimmed and weighed in order to compare with the water content of fresh leaves. The water content was measured at different time interval, however, post 2 h of detached leaves, transgenic plants lost 58 and 54.5% of their initial fresh weight as compared to the 70% in WT plants (Fig. 7A). The results exhibited that water loss in OE3 and OE4 lines was significantly less than WT. Similarly, approximately 2 folds higher chlorophyll content was observed in overexpressing lines OE3 and OE4 as compared to WT, which showed that overexpression of *NtHSP17.6* enhance drought tolerance in *B. napus* (Fig. 7B)

Discussion

In this research, the subcellular localization revealed the position of *NtHSP17.6* in cytosol, thus, we conjecture that

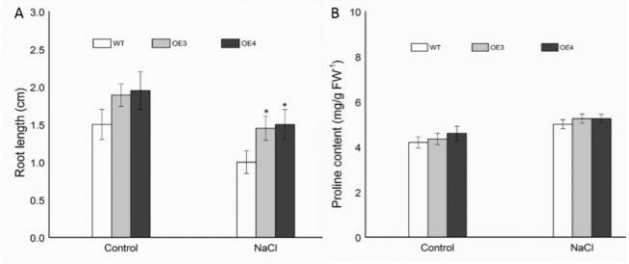

Fig. 6: *NtHSP17.6* confers salt resistance in transgenic *B. napus* **(**A) Root length of overexpressing lines was significantly longer than WT (B) Proline content was measured in transgenic *B. napus* and WT under salt stress. Single asterisk indicates significant difference compared to WT according to student t-test (*P<0.05). Vertical bar show the mean ± SD of three independent experiments

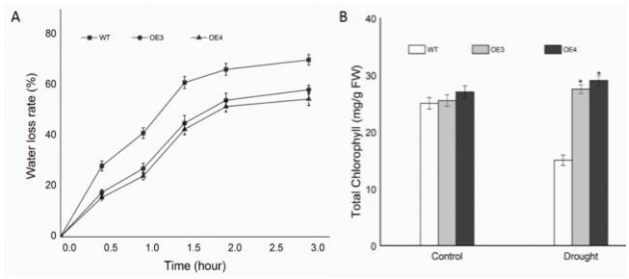

Fig. 7: Expression analysis of *B. napus* overexpressing *NtHSP17.6* under drought stress

Measurement of water loss rate of detached leaves of *B. napus* overexpressing *NtHSP17.6* and WT. Leaves weight were determined under mentioned time intervals (B) Measurement of chlorophyll content. Data represents are mean ± SD from three independent biological experiments. According to student t-test, the asterisks on the top of the columns indicate significantly differences at (*P < 0.05; **P < 0.01)

NtHSP17.6 may function in cytosol. The sHPSs have been found across a broad range of organisms (Waters 2012). *AtHSP21* and *OsHSP18.0* were located in the chloroplast and cytoplasm/nucleus, respectively (Chen *et al.* 2017; Kuang *et al.* 2017). Past comprehensive studies revealed that constitutive overexpression of sHSPs in plants are mainly correlated with increased resistance to abiotic stress (Zhang *et al.* 2014), such as constitutive expression of *sHSP17.7* in rice and *RcHSP17.8* in *Arabidopsis* strongly enhanced tolerance against heat, UV-B and several stresses, respectively (Murakami *et al.* 2004; Jiang *et al.* 2009). Overexpression of wheat *sHSP26*, *Malus sieversii MsHSP16.9* and pepper *CaHSP16.4* in *Arabidopsis* separately, developed respective plants with enhance heat tolerance (Chauhan *et al.* 2012; Yang *et al.* 2017; Huang *et al.* 2019). Similarly, overexpression of *PtHSP17.8* in *Arabidopsis* and *OsHSP18.0* in rice enhanced heat and salt resistance in plants respectively (Li *et al.* 2016b; Kuang *et al.* 2017).

In this study, coding sequence of *NtHSP17.6* was transformed into *B. napus* through floral dip method and overexpressed under the control of CaMV 35S promoter. The expression of *NtHSP17.6* was induced in leaves of *B. napus* under heat (42°C), cold (4°C), salt, and drought stress (Fig. 4), suggesting that there is an association between the up regulation of *NtHSP17.6* and the above-mentioned stresses. Post heat treatment, the response of *NtHSP17.6* was characterized by measuring total chlorophyll content, proline, and the rate of water loss. These results demonstrated that transgenic *B. napus* plants are more heat tolerant than WT.

Based on expression results and longer root length than WT, transgenic *B. napus* also confer resistance to salt stress. However, the expression of *NtHSP17.6* was not evidently much affected by cold stress. Under stress, the expression of *NtHSP17.6* was significantly enhanced in a short time interval and reached its highest level, which actually demonstrated the instantaneous and transient characteristic of heat response. These findings will assist in the interpretation that similar to other sHPSs, expression of *NtHSP17.6* is one of the key mechanism for the quick adjustment of plants under adverse heat stress. Compared to WT, transgenic plants produced more biomass and little exhibited loss of water under drought stress conditions. Altogether, the expression of *NtHSP17.6* in transgenic *B. napus* conferred higher heat and drought tolerance, and slight resistance towards salt stress.

Conclusion

This study reports the isolation and characterization of *NtHSP17.6* and also provides the evidence that constitutive expression of *NtHSP17.6* in *B. napus* confers high stress tolerance, which significantly induced under heat and drought stress. These outcomes presented that *NtHSP17.6* is a potential candidate for genetic engineering to develop crops that confer high tolerance especially against heat and drought stress.

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