Introduction of *Arabidopsis*'s heat shock factor *HsfA1d* mitigates adverse effects of heat stress on potato (*Solanum tuberosum* L.) plant

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Abstract

Thermal stress induces a wide array of morphological and physiological changes in potato affecting its development and economic yield. Response to thermal stress in plants is mostly regulated by heat shock factors (*hsfs*). The current study aimed at improving heat tolerance by transforming potato plant with heat shock factor, *HsfA1d*, using *Agrobacterium*. Gateway cloning strategy was adopted for isolation of *HsfA1d* from *Arabidopsis thaliana* and cloning into plant expression vector. The target gene was introduced into potato by infecting internodal explants with *Agrobacterium* strain GV3101 carrying *pGWB402::HsfA1d* construct. Upon exposure to heat stress, the wild-type plants turned yellowish, whereas no phenotypic effect on transgenic plants was observed. Expression of *HsfA1d* in transgenic plants was increased by 5.8-fold under thermal stress compared to room temperature. Transgenic plants exhibited 6-fold increase in the expression of downstream *HSP70* under thermal stress compared to wild-type plants. Both chlorophyll **a** and **b** were significantly decreased in wild-type plants while no such decrease was recorded in transgenic plants under thermal stress. Heat stress was found to have no significant effect on carotenoid pigments of both wild-type and transgenic plants. Significantly lower electrolyte leakage from transgenic plants was witnessed compared to wild type upon exposure to thermal stress. Transgenic plants accumulated significantly higher proline content compared to wild-type plants under heat stress. It is concluded that *HsfA1d* plays a vital role in plant thermotolerance and hence can be effectively used to enhance the resistance of crop plants against heat stress.

Keywords Potato · Thermotolerance · Heat shock factors · *HsfA1d* · *Solanum tuberosum*

Key message: Introduction of *Arabidopsis*'s *HsfA1d* confer resistance to potato plant against thermal stress by accumulating higher proline content and decreasing damage to photosynthetic pigments and plasma membrane, compared to wild plants

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Introduction

Potato, a starchy tuberous crop, is globally consumed as a food and provides raw material to agro-based industries. Potato best grows in frost-free cool seasons and is highly prone to heat stress (Hijmans 2003). The temperature range for the aboveground part of the potato plant is 20–25 °C whereas the optimal range for tuberization 15–20 °C (Van Dam et al. 1996). Elevated temperature induces a variety of changes in potato plants, affecting its development and reducing economic yield. Under thermal stress, the partitioning of photoassimilate to tubers is significantly reduced which greatly affects tuberization potential of the plant (Lafta and Lorenzen, 1995). Extensive denaturation and aggregation of cellular proteins is induced under heat stress, which if not addressed properly may result in cell death. The survival of cell under stress is primarily dependent on structure and function of the intracellular proteins (Wang et al. 2004).

The plants produce heat-shock proteins (*HSPs*) upon exposure to heat stress. On the basis of their molecular weights, *hsp*s are categorized into five groups; (1) *HSP60*, (2) *HSP70*, (3) *HSP90*, (4) *HSP100*, and (5) small heat shock proteins (*sHSPs*) (Datta *et al.* 2017). The principal role of *HSPs*, during heat stress, is to restrict aggregation of the cellular proteins. Heat shock transcription factors (*HSFs*) act as main player in regulating expression of the *HSPs* (Scharf *et al.* 2012). Under thermal stress, *HSF* is shifted to the nucleus and phosphorylated to form activated trimers. The trimeric *HSF* binds to promoter sequence of the *HSP* gene known as heat shock elements (*HSEs*). Binding of the *HSF* to *HSE* results in the generation of *HSPs* (Lata and Prasad, 2011). The existing cellular proteins and newly synthesized *HSPs* stabilize the unfolded proteins. Though, 27 *StHSFs* in the potato genome operating under adverse conditions have been identified (Tang *et al.* 2016), potato is perceived as heat-sensitive crop. On the other hand, *Arabidopsis thaliana* has been reported to synthesize 21 *HSFs* (Nover *et al.* 2001). Among these, *HsfA1d* has been found to involve in acquisition of plant thermotolerance (Hsiang-chin and Yee-yung, 2012)

The nucleotide sequence of *HsfA1d* isolated from *Arabidopsis thaliana* has shown least homology (52%) with its homologs in potato genome. To address the negative impact of thermal stress on growth and production of potatoes, research has been focussed on the development of thermotolerant potatoes using different genetic approaches. High degree of sterility and complexity of genome make potato less appealing for conventional breeding (Romano *et al.* 2003). Thus, genetic engineering provides valuable tools for improvement of potato germplasm against the heat stress (Trapero-Mozos *et al.* 2017). In the current study, *HsfA1d* was isolated from *Arabidopsis thaliana* and transformed in potato for enhancement of its thermotolerance.

Materials and methods

Preparation of entry clone

Total RNA from leaves of *Arabidopsis thaliana* (Col-O) was isolated using RNeasy® mini kit (Qiagen, Germany), and followed by treating with DNase-I (Wiame *et al.* 2000). Complementary DNA (cDNA) was then generated from the total RNA using the Super Script Transcriptase III Kit (Life Technologies, CA, USA) and the oligo (dt) primers according to the supplier's instructions. The cDNA was used as a template and denatured at 94 °C for 2 min before the PCR-based amplification. The *AtHsfA1d* was amplified with gene-specific primers using the newly synthesized cDNA as template.

The double digestion of the *pUC57GW-CmRcdB* with the *NcoI* and *XhoI* resulted in the formation of linearized vector.

The linearized vector (2804 bp) was purified with QIAquick® gel extraction kit (Qiagen, Germany). *HsfA1d* was infused in the linearized vector (*pUC57GW*) using In-Fusion® cloning kit (Clontech, USA). Highly competent *stellar* cells (Clontech, USA) were transformed with the cloning product using heat shock method. Plasmid was extracted from the colonies using QIAprep spin miniprep kit. The restriction enzymes, *NcoI* and *BglII*, were used for confirmation of the entry clone. Clustal omega and APE software were used for sequence alignment. *HsfA1d* was sub-cloned from the entry clone (*pUC57GW-HsfA1d*) into plant expression vector (*pGWB402Ω*) using *LR* clonase (Invitrogen Life Technology, USA). *Agrobacterium tumefaciens* GV3101 were transformed with *pGWB402Ω-HsfA1d* following heat shock method.

Agrobacterium-mediated plant transformation

Explant preparation

Potato tubers were grown in soil for 3 weeks. Nodal sections were excised and sterilized with 10% bleach followed by rinsing with autoclaved water. The sterilized nodal parts were grown on hormone-free MS media for 3 weeks to develop in vitro plantlets. Internodal sections of plantlets were used as explants for agro-infection.

Inoculum preparation

The *Agrobacterium tumefaciens* GV3101 harboring the plasmid *pGWB402Ω-HsfA1d* were allowed to grow overnight in LB broth containing spectinomycin (50 mg L⁻¹) at 28 °C. The resultant bacterial culture was allowed to grow for another 3 h after the addition of 45 mL fresh LB broth. The culture was centrifuged, and the pellet was washed twice with 20 mL MMA wash buffer. Finally, the inoculum was prepared by re-suspending the pellet in MS media with OD adjusted to 0.6 (OD₆₀₀ = 0.6). Acetosyringone (100 μM) was added just before infecting the explants.

Explant transformation and regeneration

Transgenic potato plants were developed by following the methods of Jung *et al.* (2005) with some modifications. Potato internodes were infected with the inoculum of *Agrobacterium* GV3101 for 10 min at 90 rpm and 22 °C. The infected explants were co-cultivated on MS media containing acetosyringone (100 μM) for 3 days in dark. The explants were transferred to MS media with zeatin riboside (ZR) (0.8 mg L⁻¹), 2, 4-D (2.0 mg L⁻¹), kanamycin (100 mg L⁻¹) and grown under 16 h light/8 h dark at 24 ± 2 °C for 4 weeks. The calli were shifted to MS with ZR (0.8 mg L⁻¹) and GA₃ (2.0 mg L⁻¹) for shoot induction. The shoots were grown on MS containing sucrose (60 mg L⁻¹), kinitin (2.5 mg L⁻¹), and

gelrite (2 mg L⁻¹), for 1 month under complete dark to induce in vitro tuberization. The in vitro tubers were grown in autoclaved 2B soil for 3 weeks to produce seedling.

Confirmation of the transgenic plants

Genomic DNA from the putative transgenic plants was isolated using Qiagen DNeasy plant mini kit and amplified through PCR with the gene-specific primers (*HsfA1d*) following the optimized PCR conditions: 2 min initial denaturation at 95 °C (1 cycle), denaturation at 95 °C for 30 s (30 cycles), annealing at 55 °C for 30 s (30 cycles) and extension at 72 °C for 1.5 min (30 cycles) with a final extension at 72 °C for 5 min. Sequence of the primers used for the detection of transgene (*HsfA1d*) was as follows:

HsfA1d-F: 5-GCCGCCTTCACCATGGATGTGAGCAA AGTAACCAC-3

HsfA1d-R: 5-CTGGGTCACCCTCGATCAAGGATTT GCCTTGAGGGATC-3

Heat treatment

The T₁ plants were exposed to heat at 42 °C for 1 week in growth chamber. The effect of heat treatment was observed phenotypically, and samples were collected for the following expression and physiological analysis:

Expression analysis of transgenic plants

Total RNA from transgenic and wild-type plants were extracted as described in Qiagen RNeasy plant mini kit. RNA was first treated with DNase (Wiame *et al.*, 2000) and then converted into first-strand cDNA using Super Script Transcriptase III Kit (Life Technologies, CA, USA) and the oligo (dt) primers. Reaction mixture for expression analysis was prepared by adding, SYBR Green (5 µl), cDNA (2 µl), 0.3 µl each of forward and reverse primers and water (2.4 µl) to a centrifuge tube. The thermal profile applied was consist of initial denaturation 98 °C for 2 min, followed by 42 cycles, with each one having denaturation 98 °C for 10 s, annealing 55 °C for 30 s, extension 72 for 2 min. Finally, 72 °C for 10 min was employed.

Physiological analysis of transgenic plants

Determination of photosynthetic pigments

Lichtenthaler and Wellburn (1983) method was followed for determination of chlorophyll “a”, “b” and total carotenoids. Leaf sample (25 mg) was mixed with magnesium oxide (MgO, 25 mg) followed by the addition of methanol (5 ml) and homogenizing on shaker for 2 h. The resultant extract was exposed to centrifugation for 5 min using 4000 rpm at normal

temperature. Absorbance of the supernatant was recorded at three different wave lengths (470 nm, 653 nm, and 666 nm) against a solvent blank using spectrophotometer (UV-2600).

The following formulas were used for calculation of photosynthetic pigments.

$$\text{Chlorophyll } a = 15.65 A_{666} - 7.340 A_{653}$$

$$\text{Chlorophyll } b = 27.05 A_{653} - 11.21 A_{666}$$

$$\text{Total carotenoids} = (1000 A_{470} - 2.860 \text{ Chla} - 129.2 \text{ Chlb}) / 245$$

Measurement of electrolyte leakage (EL)

The method of Bajji *et al.* (2002) with slight modifications was used for measurement of EL. Ten pieces (1 cm) each from three different seedlings of both transgenic and wild-type plants were collected, after heat treatment. The leaf samples were washed three times and placed in deionized water (10 mL). The conductivity recorded at the start of rehydration period was denoted as EC_i. The tubes containing the leaf segments were kept at normal room temperature for 24 h and final conductivity was measured as EC_f. The samples were autoclaved followed by cooling at 25 °C and used for measurement of total electrical conductivity (EC_t). The following equation was used to calculation of electrolyte leakage:

$$\text{EL (\%)} = (\text{EC}_f - \text{EC}_i) / (\text{EC}_t - \text{EC}_i) \times 100$$

Determination of proline

Proline content was measured by using literature methods (Bates *et al.* 1973). Fresh leaf samples (0.5 g) were homogenized in 5 mL sulfosalicylic acid (3%) and centrifuged at 4000 rpm for 30 min. The supernatant was separated, and 1 mL each of glacial acetic acid and ninhydrin reagent were added to the supernatant (1 mL). The mixture was heated (100 °C) in oven for 1 h and then shifted to ice bath for reaction stoppage. Two milliliters toluene was added to the reaction product and kept at 25 °C until the appearance of 2 layers. Absorbance of the upper colored layer was recorded at 520 nm. Proline content was measured by following the formula:

$$\text{Proline } (\mu\text{M/g FW}) = [(\mu\text{g proline/mL} \times \text{mL toluene}) / 115.5 \mu\text{g } \mu\text{mol}^{-1}] / [\text{g sample}/5].$$

Results

Total RNA from leaves of *Arabidopsis thaliana* (Col-0) was extracted and used for the synthesis of full-length complementary DNA (cDNA). The expected 1458 bp *HsfA1d* was produced when full-length complementary DNA (cDNA) of *Arabidopsis thaliana* was used as a template (Sup. 1a). Digestion of cloning vector, *pUC57GW-CmRcdB* with

restriction enzymes, *XhoI* and *NcoI* resulted in 2804, 833, and 630 bp expected fragments (Sup. 1b). The insert (*AtHsfA1d*) was infused in linearized vector (purified *pUC57GW*) and successfully transformed into *stellar* competent cells. Digestion of entry clone with restriction enzymes, *NcoI* and *BglII*, resulted in four fragments (2828 bp, 807 bp, 391 bp, and 237 bp) while two fragments (3637 bp and 630 bp) were produced for empty vector with same set of enzymes (Sup. 1c). Sequence analysis with M13 primers revealed 100% homology of the entry clone with *AtHsfA1d* (Sup. 2). The newly synthesized entry clone has been shown in the Sup. 3.

Heat shock transcription factor, *HsfA1d*, was sub-cloned in plant expression vector (*pGWB402Ω*), followed by transformation to *stellar* competent cells (Sup. 4). The plasmid (*pGWB402Ω-HsfA1d*) extracted from transformed bacterial colonies produced three expected fragments (8665 bp, 2439 bp, and 391 bp) upon cleavage with *NCOI*, while digestion of *pGWB402Ω* (control) with the same restriction enzyme yielded two bands (9384 bp and 2294 bp) with the same enzyme (Sup. 5a). *HsfA1d* sub-cloned in plant expression vector has been shown in Sup. 5b. After restriction digestion-based confirmation, *Agrobacterium* GV3101 cells were successfully transformed with *pGWB402Ω-HsfA1d* (Sup. 6). Potato internodes infected with *Agrobacterium* harboring *pGWB402Ω-HsfA1d* exhibited growth on kanamycin containing media while no such growth was observed in wild type and ultimately died on selection media (Sup. 7a). Calli were produced, after 4 weeks, on MS media supplemented with ZR trans-isomer (0.8 mg L^{-1}) and 2,4-D (2.0 mg L^{-1} ; Sup. 7b). In 3 weeks, the calli started regeneration on MS media supplemented with ZR trans-isomer (0.8 mg L^{-1}) and GA_3 (2.0 mg L^{-1} ; Sup. 7c). The regenerated shoots produced in vitro tubers on MS media containing sucrose (60 mg L^{-1}), kinitin (2.5 mg L^{-1}) and gelrite (2.0 mg L^{-1}) in 1 month under complete darkness (Sup. 7d). Expected 1458 bp band was observed when transgenic DNA of putative transgenic plants was exposed to PCR with *HsfA1d*-specific primers while no such band was produced when DNA extracted from wild-type plants was used as template (Sup. 8).

Upon heat treatment, no obvious adverse effect on growth of transgenic plants was observed at phenotypic level while wild type showed stunted growth and turned yellowish (Fig. 1). The expression of target gene (*HsfA1d*) was witnessed in transgenic plants both at room temperature and thermal stress; however, the level of expression was more pronounced (5.5-fold) upon exposure to heat stress compared to room temperature (Fig. 2a). Transgenic plants exhibited 4- and 6-fold higher expression of *HSP70* at room temperature and thermal stress respectively, compared to wild-type plants (Fig. 2b). Both transgenic and wild-type plants exhibited 2.2-fold increase in the expression *HSP90* under heat stress compared to room temperature; however, no significant difference in their expression was recorded at room temperature (Fig. 2c).

Fig. 1 Phenotypic effect of heat treatment (42°C) on wild type (WT) and transgenic potato (TP)

Heat stress caused significant reduction in chlorophyll *a* content of wild-type plants compared to room temperature while no such decline was recorded in transgenic plants (Fig. 3a). Significant decrease in chlorophyll *b* content of wild-type plants was observed upon exposure to thermal stress. On the other hand, no significant decrease in chlorophyll *b* content of transgenic plants was recorded under heat stress (Fig. 3b). Both wild-type and transgenic plants revealed no significant difference in their carotenoid pigments under thermal stress and room temperature (Fig. 3c). Wild-type and transgenic plants exhibited no significant difference in their EL at room temperature; however, highly significant and significant increase in EL of wild-type and transgenic plants, respectively, was recorded upon exposure to heat stress (Fig. 3d). Thermal stress induced significant increase in proline content of wild-type plants as compared to room temperature. Similarly, transgenic plants exhibited highly significant increase in their proline contents under thermal stress. However, both types of plants showed no significant difference in accumulated proline at room temperature (Fig. 3e).

Discussion Genetic engineering has provided a valuable tool in hands of molecular biologists to enhance crop tolerance against different stresses that ultimately leads to increase productivity. The knocking out of *HsfA1d* from *Arabidopsis* plants remarkably declines their thermotolerance (Hsiang-chin and Yee-yung, 2012). Similarly, *HsfA1d* overexpression has significantly enhanced the ability of model tobacco plant to counter the detrimental effects of heat stress (Shah et al. 2017). The idea behind the current study was to investigate whether overexpression of *HsfA1d* enhance thermotolerance of the crop plant like potato. *Agrobacterium*-mediated transformation was used to generate transgenic potato plants overexpressing *HsfA1d*. Upon exposure to heat stress, wild-type plants showed reduced growth while transgenic plants exhibited more tolerance and hence no significant adverse effects on

Fig. 2 Effect of heat treatment on expression of target and downstream genes belong to wild type (WT) and transgenic plants (T.P). Data are shown as means of control and treatments from 3 independent replicates. Different letters indicate that the change is statistically significant as

determined by 2 factorial ANOVA followed by LSD ($p \leq 0.05$). Effect of heat treatment on expression of **a** *HsfA1d*, **b** *HSP70*, and **c** *HSP90* of wild type (WT) and transgenic plants (TP)

growth were recorded. The transgene (*HsfA1d*) incorporated in potato genome enabled the plantlets to remain green under thermal stress, while the wild-type plants could not cope with the harsh effects of heat stress and turned yellowish (Fig. 1).

The higher expression (5.5-fold) of target gene exhibited by transgenic plants under thermal stress (Fig. 2a) was in line with Qain *et al.* (2014). These results further endorsed the findings of Xue *et al.* (2010) that heat stress causes upregulation of transgene. The elevated expression of *HSP70* recorded in transgenic plants at room temperature (4-fold) strengthened the reports of Higashi *et al.* (2013) that transgenic *Arabidopsis* overexpressing *TsHsfA1d* caused upregulation of many genes under normal growth temperature. Such results further

ascertained the role of *HsfA1d* as transcription activator. The increase in expression of *HSP70* under thermal stress (6-fold; Fig. 2b) was in agreement with Qain *et al.* (2014) that heat stress induces the expression of heat shock genes. These results showed that *HsfA1d* triggers the expression of downstream *HSP70* more than *HSP90* (Fig. 2c) at transcription level. The significant decrease in both chlorophyll **a** and **b** content exhibited by wild-type plants, under thermal stress, is indicative of heat-induced structural damage to the chloroplast. The decline in chlorophyll content, as recorded in Fig. 3a, b, has also previously been reported by Naz *et al.* (2018) in potato. The higher chlorophyll (**a**, **b**) retained by transgenic plants compared to wild type is in agreement with Xu *et al.*

Fig. 3 Effect of heat treatment on different parameters of wild type (WT) and transgenic plants (T.P). Data are shown as means of control and treatments from 3 independent replicates. Different letters indicate that the change is statistically significant as determined by 2 factorial ANOVA

followed by LSD ($p \leq 0.05$). Effect of heat treatment on **a** chlorophyll 'a' content, **b** chlorophyll 'b' content, **c** carotenoids, **d** electrolyte leakage, and **e** proline content of wild type and transgenic plants

(2014). No effect of heat stress on carotenoids (Fig. 3c) content reflects their heat-stable nature and is in line with study of Mohamed and Aisha (2019). The magnitude of electrolyte

leakage gives excellent clue about damage that happened to the plant cell membrane (Bajji *et al.* 2002). Thermal stress has enhanced the electrolyte leakage of both types of plants;

however, the extent of leakage was more pronounced in wild-type than transgenic plants as reflected in Fig. 3d. The lipid component of plasma membrane is particularly prone to thermal stress widening pores in cell membrane of wild-type plants associated with more leakage. In contrast, the cell membrane of transgenic plants was found to offer more protection against the damage caused by thermal stress leading to less electrolyte leakage (Shah *et al.* 2017). The low level of EL shown by transgenic plants under thermal stress is also reported by Mohamed and Aisha (2019). Proline serves as excellent stress marker. The obvious increase in proline content of transgenic plants compared to wild type, in response to thermal stress (Fig. 3e), has strengthened the previous reports of Xue *et al.* (2010). It has been reported that enhanced accumulation of proline acts as protectant against thermal stress and reduce the damage to the cellular machinery (Gupta *et al.* 2013).

Author's contribution Research work was conducted by Zamarud Shah under the supervision of Safdar Hussain Shah and Gul Shad Ali. Iqbal Munir and Asad Jan checked the manuscript time to time for improvement. Nisar Ahmed helped in figure setting. Raham Sher Khan and Arshad Iqbal contributed in revising the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Bajji M, Kinet J, Lutts S (2002) The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regul* 36:61–70
- Bates L, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. *Plant Soil* 39:205–207
- Datta K, Rahalkar K, Dinesh DK (2017) Heat Shock Proteins (Hsp): Classifications and its involvement in health and disease. *J Pharma Care Health Sys* 4(2):1–3
- Gupta NK, Agarwal S, Agarwal VP, Nathawat NS, Gupta S, Singh G (2013) Effect of short-term heat stress on growth, physiology and antioxidative defense system in wheat seedlings. *Acta Physiol Plant* 35:1837–1842
- Lichtenthaler HK, Wellburn AR (1983) Determination of total carotenoids and chlorophyll a and b of leaves and different solvents. *Biochem Soc Trans* 11(5):591–592
- Higashi Y, Ohama N, Ishikawa T, Katori T, Shimura A, Kusakabe K, Yamaguchi K, Shinozaki JJ, Tanaka M, Seki M, Shinozaki K, Sakata Y, Hayashi T, Taji T (2013) *HsfA1d*, a protein identified via FOX hunting using *Thellungiella salsuginea* cDNAs improves heat tolerance by regulating heat-stress-responsive gene expression. *Mol Plant* 6(2):411–422
- Hijman JB (2003) The effect of climate change on global potato production. *Amer J Pot Res* 80:271–280
- Hsiang-chin L, Yee-yung C (2012) Acquired thermotolerance independent of heat shock factor A1 (*HsfA1*), the master regulator of the heat stress response. *Plant Signal Behav* 7(5):547–550
- Jung CS, Griffiths HM, De-Jong DM, Cheng S, Bodis M, De-Jong WS (2005). The potato P locus codes for flavonoid 3' 5'-hydroxylase. *Theor Appl Genet* 110(2):269–275
- Lafta AM, Lorenzen J (1995) Effect of high temperature on plant growth and carbohydrate metabolism in potato. *Plant Physiol* 109(2):637–643
- Lata C, Prasad M (2011) Role of DREBs in regulation of abiotic stress responses in plants. *J Exp Bot* 62:4731–4748
- Mohamed AE, Aisha AA (2019) Overexpression of rice Rab7 gene improves drought and heat tolerance and increases grain yield in rice (*Oryza sativa* L.). *Genes (Basel)* 10(1):56
- Naz N, Durrani F, Shah Z, Khan NA, Ullah I (2018) Influence of heat stress on growth and physiological activities of potato (*Solanum tuberosum* L.). *Phyton* 87:225–230
- Nover L, Bharti K, Döring P, Mishra SK, Ganguli A, Scharf KD (2001) *Arabidopsis* and the heat stress transcription factor world: how many heat stress transcription factors do we need. *Cell Stress Chaperones* 21:61–77
- Qain J, Chen J, Liu YF, Yang LL, Li WP, Zhang LM (2014) Overexpression of *Arabidopsis HsfA1a* enhances diverse stress tolerance by promoting stress-induced *HSP* expression. *Genet Mol Res* 13(1):1233–1243
- Romano A, Raemaker K, Bernardi J, Visser R, Mooibroek H (2003) Transgene organization in potato after particle bombardment mediated co-transformation using plasmid and gene cassettes. *Transgenic Res* 12:461–473
- Scharf KD, Berberich T, Ebersberger I, Nover L (2012) The plant heat stress transcription factor (*Hsf*) family: structure, function and evolution. *Biochim Biophys Acta* 1819:104–119
- Shah Z, Safdar HS, Asad J, Gul SA (2017) Overexpression of heat shock transcription factor *HsfA1d* enhances thermotolerance of tobacco plants. *Sarhad J Agric* 33(1):162–170
- Tang R, Zhu W, Song X, Lin X, Cai J, Wang M (2016) Genome-wide identification and function analyses of heat shock transcription factors in potato. *Front Plant Sci* 7:490
- Trapero-Mozos A, Wayne LM, Laurence JMD, Karen M, Jennifer S, Lesley T, Glenn JB, Robert DH, Mark AT (2017) Engineering heat tolerance in potato by temperature-dependent expression of a specific allele of Heat-Shock Cognate 70. *Plant Biotechnol J* 16(1):197–207
- Van Dam J, Koosman PL, Struik PC (1996) Effect of temperature and photoperiod on early growth and final number of tubers (*Solanum tuberosum* L.). *Potato Res* 39:51–62
- Waime I, Remy SR, Saqi L (2000) Irreversible heat inactivation of DNase I with RNA degradation. *Biotech* 29(2):252–256
- Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of heat shock protein and molecular chaperones in the abiotic stress response. *Trends Plant Sci* 9(5):244–252
- Xue Y, Peng R, Xiang A, Li X, Zha D, Yao Q (2010) Overexpression of heat shock protein gene *hsp26* in *Arabidopsis thaliana* enhances heat tolerance. *Biol Plant* 4(1):105–111
- Xu Q, Xu X, Shi Y, Xu J, Huang B (2014) Transgenic tobacco plants overexpressing a grass *PpEXP1* gene exhibit enhanced tolerance to heat stress. *PLoS One* 9(7):96–102

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