Differential Expression Gene/Protein Contribute to Heat Stress-Responsive in Tetraena propinquaa in Saudi Arabia

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Research article

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Abstract

Background

Within their natural habitat, plants are subjected to abiotic stresses that include heat stress. In the current study, the effect of 4h, 24h and 48h of heat stress on *Tetraena propinqua* ssp. *migahidii* seedling's protein profile and proteomic analyses were investigated.

Results

Total soluble protein SDS-PAGE profile showed 18-protein bands downregulated at 4h and 48h, however, 20-protein bands were upregulated at 24h of heat stress. A proteomic analysis showed that 81 and 59 targets are involved in gene and protein expression respectively.

Conclusions

The genes and proteins involved in transcription, translation, photosynthesis, transport and other unknown metabolic processes, were differentially expressed under treatments of heat stress. These findings provide insights into the molecular mechanisms related to heat stress, in addition to its influence on the physiological traits of *T. propinqua* seedlings. Heat stress mediated differential regulation genes indicate a role in development and stress response of *T. propinqua*. The candidate dual specificity genes identified in this study paves way for more molecular analysis of up- and down-regulation.

Background

The natural environment for plants involves a series of abiotic and biotic stresses. The plants response to these stresses are combinations of changes in various processes [1]. Among the common abiotic stresses, high temperature is considered a major factor that substantially affect plant growth and developmental processes [2]. Heat stress creates changes in physiological processes, such as increased lipid peroxidation, which decreases the thermal stability of the cell membrane [3] and excess generation of (ROS reactive oxygen species), which produce oxidative stress [4]. Heat stress also induces production of compatible solutes which are able to organize proteins and cellular structures, to maintain cell turgor by osmotic adjustment, and alter the antioxidant system to return the cellular redox balance and homeostasis [5]. At the gene expression levels, heat stress is associated with multiple processes involving upregulation and downregulation of genes controlling various proteins particularly heat shock proteins (HSPs), transcription factors (TFs), and other stress related genes [6]. These genes are in control of the expression of osmo-protectants, detoxifying enzymes, transporters, and regulatory proteins involved in essential processes like photosynthesis [7] and changes in protein domains, and gene and protein structures [8].

Proteomics is defined as the large-scale study of proteins. A proteome is a set of proteins generated in an organism or biological system. The proteome varies from cell to cell and alters over time. Additionally, protein activity is also modified by various factors in addition to the expression level of the relevant gene [9, 10]. Proteomics discover heat stress-responsive proteins (HRPs) and explain pathways which are critical for heat tolerance [11]. Various proteins linked to signal transduction, photosynthesis, antioxidant defense, transcriptional and post-transcriptional regulation, protein synthesis and turnover, carbohydrate and energy metabolism play vital roles in preserve leaves against heat stress [5, 12, 13]. Proteomic responses to heat stress was investigated in *Arabidopsis* leaves [14], maize [15], spinach [16], perennial ryegrass (*Lolium perenne*) [11], *Rhazya stricta* [17], grape [18]. Proteomics can be done by optimizing the sample preparation as well as by the LC-MS method for specific proteins [19]. A number of programs for protein identification take the output of peptide sequences from mass spectrometry and get back information about matching identical proteins. This is done during algorithms which perform alignments with proteins from famous databases such as UniProt [20] to predict proteins in the sample with surety.

*Tetraena. propinqua* (Decne.) Ghaz. & Osborne is a species of *Tetraena* distributed in Sinai of Egypt, Arabian Peninsula of Saudi Arabia and Yemen, Iraq, S Iran, Afghanistan and Pakist [21]. It belongs to subfamily *Zygophyllaceae*; the largest of family *Zygophyllaceae* [22], which includes about 180 species of shrubs, subshrubs and herbs mainly distribute in arid and semi-arid areas mostly in tropics and subtropics saline regions. Alzahrani [23] reported that *Tetraena propinqua* ssp. *propinqua*, and *Tetraena propinqua* ssp. *migahidii* are a new subspecies combination nova from Saudi Arabia in this subfamily.
In the present study, we examine the influence of heat stress on protein expression using proteomic methods in *T. propinqua* seedlings exposed to different durations of high temperature stress, with the goal of identifying proteins closely related to heat stress which enables *T. propinqua* to adapt to heat tolerance in Saudi Arabia.

**Results**

**SDS-PAGE Profile of Total Soluble Proteins**

The protein pattern of *T. propinqua* seedlings in one-dimensional polyacrylamide gel electrophoresis (SDS-PAGE) showed no remarkable differences (Fig. 1). The relative expression of 18 protein bands (with molecular weights of about 102.3, 93.8, 71.4, 66, 55, 53.2, 47.1, 41.3, 40.2, 38.4, 35.1, 32, 26.8, 25, 10.9, 10.5, 10, 9.4 KD) appeared under the control and heat treatments. The relative intensity of these bands were often downregulated after 4 h and 48 h at high temperature, while upregulated after 24 h of high temperature. The expression of protein band (with molecular weights of about 86.5 and 30.2 KD) newly appeared at 24 h of high temperature. On the other hand, protein band of 31.4 KD appeared at 48 h under normal conditions then disappeared under high temperature of 48 h.

**Heat Stress-Responsive Proteome in T. propinqua**

In total, 931 protein species and 81 gene at germinating stage were identified. After 4 hours of germination, 110 protein were identified in C4 (control plants) and 173 protein scored in T4 (treated plants). However, after 24 h, 181 protein were identified in C24 (control) decreased to 151 protein in T24 (treated). After 48 h, 136 protein were identified in C48 (control) increased to 180 protein in T48 (treated). Eighty-one genes were identified in control and treated *T. propinqua* seedlings depended on appearance and disappearance of proteins they code for (Table 1).
Table 1
List of expressed genes by heat stress in *T. propinqua* seedlings depending on appearance and disappearance under heat stress treatments using MALDI-Triple-TOF.

| No. | Gene | Description | C4 | T4 | C24 | T24 | C48 | T48 |
|-----|------|-------------|----|----|-----|-----|-----|-----|
| 1.  | rpoA | DNA-directed RNA polymerase subunit alpha | 1  | 1  | 1   | 1   | 1   | 1   |
| 2.  | rpoC1| DNA-directed RNA polymerase subunit beta’ | 1  | 1  | 1   | 1   | 1   | 1   |
| 3.  | rpoC2| DNA-directed RNA polymerase subunit beta” | 1  | 1  | 1   | 1   | 1   | 1   |
| 4.  | rpoB | DNA-directed RNA polymerase subunit beta  | 1  | 1  | 1   | 1   | 1   | 1   |
| 5.  | rpb2 | DNA-directed RNA polymerase subunit B     | 1  | 0  | 0   | 0   | 1   | 1   |
| 6.  | WRKY21 | WRKY transcription factor 21 | 1  | 1  | 1   | 1   | 1   | 1   |
| 7.  | matK | Maturase K                                   | 1  | 1  | 1   | 1   | 1   | 1   |
| 8.  | matR | Maturase                                    | 1  | 1  | 1   | 1   | 1   | 1   |
| 9.  | EMB2765 | Embryo defective 2765                  | 1  | 1  | 1   | 1   | 0   | 1   |
| 10. | PHYC | Phytochrome C                               | 1  | 1  | 1   | 0   | 0   | 1   |
| 11. | rpl14 | 50S ribosomal protein L14, chloroplastic    | 1  | 1  | 1   | 1   | 1   | 1   |
| 12. | rpl23 | 50S ribosomal protein L23, chloroplastic   | 1  | 1  | 1   | 1   | 1   | 1   |
| 13. | rpl22 | 50S ribosomal protein L22, chloroplastic   | 1  | 1  | 1   | 1   | 1   | 1   |
| 14. | rpl20 | 50S ribosomal protein L20, chloroplastic   | 1  | 0  | 0   | 0   | 0   | 0   |
| 15. | rpl2 | 50S ribosomal protein L2, chloroplastic    | 1  | 1  | 1   | 1   | 1   | 0   |
| 16. | rpl16 | 50S ribosomal protein L16, chloroplastic   | 0  | 1  | 1   | 0   | 0   | 1   |
| 17. | rpl36 | 50S ribosomal protein L36, chloroplastic   | 0  | 0  | 1   | 0   | 0   | 0   |
| 18. | rpl32 | 50S ribosomal protein L32, chloroplastic   | 1  | 1  | 1   | 1   | 1   | 1   |
| 19. | rpl33 | 50S ribosomal protein L33, chloroplastic   | 0  | 1  | 0   | 0   | 0   | 1   |
| 20. | rps18 | 30S ribosomal protein S18, chloroplastic   | 0  | 1  | 1   | 0   | 1   | 1   |
| 21. | rps8  | 30S ribosomal protein S8, chloroplastic    | 1  | 1  | 1   | 0   | 1   | 1   |
| 22. | rps4  | 30S ribosomal protein S4, chloroplastic    | 1  | 1  | 1   | 1   | 1   | 1   |
| 23. | rps19 | 30S ribosomal protein S19, chloroplastic   | 1  | 0  | 0   | 1   | 1   | 0   |
| 24. | rps7  | 30S ribosomal protein S7, chloroplastic    | 1  | 0  | 1   | 1   | 0   | 0   |
| 25. | rps11 | 30S ribosomal protein S11, chloroplastic   | 0  | 1  | 1   | 1   | 1   | 1   |
| 26. | rps14 | 30S ribosomal protein S14, chloroplastic   | 0  | 0  | 1   | 0   | 0   | 0   |
| 27. | rps2  | 30S ribosomal protein S2, chloroplastic    | 1  | 1  | 1   | 1   | 0   | 1   |
| 28. | rps15 | 30S ribosomal protein S15, chloroplastic   | 1  | 0  | 1   | 1   | 1   | 1   |
| 29. | rps3  | Ribosomal protein S3                       | 1  | 1  | 1   | 1   | 1   | 1   |
| 30. | petA  | Cytochrome f                                | 1  | 1  | 1   | 1   | 1   | 1   |
| 31. | petD  | Cytochrome b6-f complex subunit 4           | 1  | 1  | 1   | 1   | 1   | 1   |
| 32. | psbE  | Cytochrome b559 subunit alpha               | 1  | 0  | 1   | 0   | 1   | 1   |
| 33. | ycf3  | Photosystem I assembly protein Ycf3         | 0  | 0  | 0   | 0   | 1   | 0   |
| 34. | ycf4  | Photosystem I assembly protein Ycf4         | 0  | 1  | 1   | 0   | 0   | 1   |
| No. | Gene   | Description                                                                 | C4 | T4 | C24 | T24 | C48 | T48 |
|-----|--------|------------------------------------------------------------------------------|----|----|-----|-----|-----|-----|
| 35. | psbL   | Photosystem II reaction center protein L                                      | 1  | 1  | 1   | 1   | 1   | 1   |
| 36. | psbI   | Photosystem II reaction center protein I                                     | 1  | 1  | 1   | 1   | 1   | 1   |
| 37. | psbK   | Photosystem II reaction center protein K                                     | 1  | 1  | 0   | 0   | 1   | 0   |
| 38. | psaA   | Photosystem I P700 chlorophyll a apoprotein A1                               | 0  | 0  | 0   | 0   | 1   |     |
| 39. | psaB   | Photosystem I P700 chlorophyll a apoprotein A2                               | 1  | 1  | 1   | 1   | 1   | 1   |
| 40. | psaC   | Photosystem I iron-sulfur center                                             | 1  | 0  | 1   | 0   | 1   | 1   |
| 41. | psbB   | Photosystem II CP47 reaction center protein                                  | 0  | 0  | 0   | 0   | 1   | 0   |
| 42. | psbH   | Photosystem II reaction center protein H                                      | 1  | 0  | 0   | 1   | 1   | 0   |
| 43. | psbD   | Photosystem II D2 protein                                                    | 0  | 0  | 1   | 0   | 0   | 0   |
| 44. | RCA1   | Ribulose bisphosphate carboxylase/oxygenase activase 1, chloroplastic       | 1  | 1  | 1   | 1   | 1   | 1   |
| 45. | RCA2   | Ribulose bisphosphate carboxylase/oxygenase activase 2, chloroplastic       | 1  | 1  | 0   | 1   | 1   | 1   |
| 46. | rbcL   | Ribulose bisphosphate carboxylase large chain                                | 1  | 1  | 1   | 1   | 1   | 1   |
| 47. | ycf2   | Hypothetical chloroplast RF21                                                 | 1  | 1  | 1   | 1   | 1   | 1   |
| 48. | atpE   | ATP synthase epsilon chain, chloroplastic                                    | 1  | 1  | 1   | 1   | 1   | 1   |
| 49. | atp1   | ATP synthase subunit alpha                                                   | 1  | 1  | 1   | 1   | 1   | 1   |
| 50. | atpB   | ATP synthase subunit beta, chloroplastic                                     | 1  | 1  | 1   | 1   | 1   | 1   |
| 51. | atpA   | ATP synthase subunit alpha, chloroplastic                                    | 1  | 1  | 1   | 1   | 1   | 1   |
| 52. | atpI   | ATP synthase subunit a, chloroplastic                                        | 1  | 1  | 1   | 1   | 0   | 0   |
| 53. | atpF   | ATP synthase subunit b, chloroplastic                                        | 1  | 1  | 1   | 0   | 1   | 1   |
| 54. | ndhE   | NAD(P)H-quinone oxidoreductase subunit 4L, chloroplastic                    | 1  | 1  | 1   | 1   | 1   | 1   |
| 55. | ndhD   | NAD(P)H-quinone oxidoreductase chain 4, chloroplastic                        | 0  | 0  | 0   | 1   | 0   | 1   |
| 56. | ndhB   | NADH-quinone oxidoreductase subunit N                                        | 0  | 1  | 1   | 0   | 1   | 1   |
| 57. | ndhF   | NAD(P)H-quinone oxidoreductase subunit 5, chloroplastic                     | 1  | 1  | 1   | 1   | 1   | 1   |
| 58. | ndhA   | NAD(P)H-quinone oxidoreductase subunit 1, chloroplastic                     | 1  | 1  | 1   | 1   | 1   | 1   |
| 59. | ndhJ   | NAD(P)H-quinone oxidoreductase subunit J, chloroplastic                     | 1  | 1  | 1   | 0   | 1   | 1   |
| 60. | ndhH   | NAD(P)H-quinone oxidoreductase subunit H, chloroplastic                     | 1  | 1  | 1   | 1   | 1   | 1   |
| 61. | ndhI   | NAD(P)H-quinone oxidoreductase subunit I, chloroplastic                     | 0  | 0  | 0   | 1   | 0   | 1   |
| 62. | ndhK   | NAD(P)H-quinone oxidoreductase subunit K, chloroplastic                     | 1  | 1  | 0   | 0   | 1   | 1   |
| 63. | ycf1   | Protein TIC 214                                                              | 1  | 1  | 1   | 1   | 1   | 1   |
| 64. | cemA   | envelope membrane protein, chloroplastic                                      | 0  | 1  | 1   | 1   | 0   | 1   |
| 65. | AKT1   | Potassium channel                                                            | 1  | 1  | 1   | 1   | 0   | 1   |
| 66. | NHX    | Vacuolar Na+/H+ antiporter                                                   | 1  | 1  | 1   | 1   | 1   | 1   |
| 67. | DGAT1a | O-acyltransferase                                                            | 0  | 0  | 1   | 0   | 0   | 0   |
| 68. | DGAT1b | O-acyltransferase                                                            | 0  | 1  | 0   | 0   | 0   | 0   |
The genes of proteins were classified by gene ontology (GO) annotation, and then classified into three functional groups: molecular function, biological process, and cellular component. The results of the GO analyses for the various treatments are shown in Table 1. Ten genes are involved in transcription, encoding rpoA, rpoC1, rpoC2, rpoB, rpb2, WRKY21, matK, matR, MB2765 and PHYC, among them rpb2 and PHYC disappeared after 4 and 24 h of heat treatment, but EMB2765 and PHYC appeared after 48 h under 40°C heat stress. 19 genes for rpl14, rpl23, rpl22, rpl20, rpl16, rpl36, rpl32, rpl33, rps18, rps8, rps4, rps19, rps7, rps11, rps14, rps2, rps15 and rps3 are involved in translation (Figs. 2). Among them, some genes for rpl and rps disappeared such as rpl20, rpl2, rpl36, rps8, rps7, rps14, rps15, however, other genes were observed like rpl33, rpl16, rps11, rps2 under different exposure time of heat stress treatments. However, some genes observed after 4 h and 48 h and disappeared after 24 h for example the rpl16 and rps18, but rps19 disappeared after 4 h and 48 h and appeared after 24 h under 40°C of heat stress in T. propinqua seedlings (Table 1).

In T. propinqua seedlings, 15 genes are involved in the transport process (Table 1, Fig. 2), among them, ycf1 is involved in protein transport, but cemA is involved in hydrogen ion transport, AKT1 involved in regulation of ion transmembrane transport, and NHX involved in regulation of pH. DGAT1a and DGAT1b involved triglyceride biosynthetic process. CAAT1 and CAAT2 involved in acyltransferase. accD involved in fatty acid biosynthetic process and malonyl-CoA biosynthetic process. ccsA and ccmB involved in cytochrome complex assembly. VP1-1, VP1-2 and clpP involved in hydrolase. Xdh involved in oxidation-reduction process. After 4 h of heat stress, cemA, DGAT1b observed, but CAAT2 disappeared. At 24 h of heat stress, DGAT1a, ccsA, VP1-2, VP1-1 disappeared. At 48 h of heat stress, cemA, AKT1, CAAT1, ccmB, VP1-1, clpP and CAAT2 appeared under 40°C with compared control. APS1, APS2, PPS1 and psbA genes were unknown function. Among them, APS7 appeared at 24 and 48 h, and PPS1 observed at 24 h, however, psbA disappeared after 48 h under 40°C of heat stress compared control. The cellular components of proteins were in chloroplast, nucleus, mitochondrion, membrane, endoplasmic reticulum, ribosome and unknown components (Fig. 3).

On the other hand, 17 genes for petA, petD, psbE, ycf3, ycf4, psbL, psbl, psbK, psaA, psaB, psaC, psbB, psbH, psbD, RCA1, RCA2 and rbcL are involved in photosynthesis and photorespiration (Table 1; Fig. 2). The proteins encoded by some genes disappeared after 4 h under 40°C of heat stress like psbE, psaC, psbH, but the expression of other genes appeared such as ycf4. After 24 h of heat stress, the expression of the psbE, ycf4, psaC and psbD genes disappeared, but psbH and RCA2 observed compared to control. At 48 h of heat stress, ycf3, psbK, psaB and psbH genes disappeared, but the protein encoded by ycf4 and psaA appeared in T.
propinqua seedlings. In *T. propinqua* seedlings, the *ycf2*, *atpE*, *atp1*, *atpB*, *atpA*, *atpF*, *ndhE*, *ndhD*, *ndhB*, *ndhF*, *ndhA*, *ndhJ*, *ndhH*, *ndhI* and *ndhK* are involved in metabolic processes such as ATP synthesis, coupled proton transport, photosynthesis and light reaction (Fig. 2). Among them, the proteins encoded by *atpF* and *ndhJ* genes disappeared after 24 h of heat stress; however, the proteins encoded by *ndhD* and *ndhI* genes appeared after 24 h, 48 h under 40°C of heat stress compared to the control. On the other hand, the protein encoded by the *ndhB* gene was observed at 4 h then disappeared after 24 h 40°C of heat stress compared to control (Table 1, Fig. 2). A PCA-biplot of the 81 gene differentially expressed in *T. propinqua* seedlings under heat stress is shown in Fig. 4.

Top 59-proteins of *T. propinqua* seedlings differentially expressed in control and heat exposed seedlings based on abundance (NSAF; protein expression) (Table 2, Fig. 5). Four proteins of DNA-directed RNA polymerase such as A0A4Y5PRE0, A0A4Y5PPR0, A0A0M3TGK1 and D3WDG9 decreased under 4 h and 48 h of heat stress; however, A0A3G4R1Z0 increased after 24 h of heat stress compared with control. The protein expression of T1Q0S2 (Maturase K) decreased after 4 h and 24 h but increased after 48 h of heat stress in *T. propinqua* seedlings. Three of putative LOV domain-containing proteins were A0A126 × 286, A0A140F7J3 and A0A140F7J1 decreased under 4 h of heat stress compared with control. The protein expression decreased after 24 h of heat stress for 20 of ribulose bisphosphate carboxylase proteins i.e. A0A0F6RA63, O20282, Q0ZQ55, O20271, B3FD36, A0A0F6TN85, A0A3G1ZJ92, B3FD32, A0A2U8T527, A9XUT7, B3FD26, A0A0F6RA65, O20257, A0A4Y5PPU8, A0A4Y5PQ6, Q9SBY1, Q9SC93, Q9SC53, Q9SBY0 and Q9SBW6. However, 16 protein increased after 24 h of heat stress; these are Q9BAD9, B3FD25, Q9TI23, Q9TI22, A0A0F6TMP4, A0A3G3NI82, A0A172C083, A0A142LSY1, O20289, O20239, A0A1U9YH05, A0A3G4R1V6, A0A0M3TGJ0, Q9SC24, A0A1S6WN95 and A0A411GWE8. After 48 h of heat stress, the protein expression increased for 27 proteins were A0A0F6RA63, A0A2Z4N431, O20271, B3FD25, O20400, A0A291L1Z1, A0A3G1ZJ92, B3FD32, A0A0F6TMP4, B3FD26, A0A0F6RA65, Q9TI21, O20289, A0A3G3NI75, Q9THY9, B3FD28, Q9BAD8, A0A3G3NI71, A0A4Y5PPU8, A0A3G4R1V6, Q9SBY1, Q9SC24, Q9SC93, Q9SBW8, Q9SBY0, Q9SBX9 and Q9SBW7. However, it decreased in photosystem I assembly protein *Ycf3* (A0A4Y5PBR9) after 4 h of heat stress compared to control. One actin protein (A7LHF2) increased after 4 h of heat stress in *T. propinqua* seedlings.
Table 2
List of top 59 differentially expressed proteins by heat stress in *T. propinqua* seedlings based on abundance (NSAF), using MALDI-Triple-TOF analysis.

| No. | Accession | Description | Gene | NSAF (Abundance) |
|-----|-----------|-------------|------|------------------|
|     | (Uni Prot KB) | | | C4 | T4 | C24 | T24 | C48 | T48 |
| 1   | A0A4Y5PRE0 | DNA-directed RNA polymerase subunit beta’ (A0A4Y5PRE0_LARTR) | rpoC1 | 0.00065 | 0 | 0 | 0 | 0.00027 | 0 |
| 2   | A0A3G4R1Z0 | ~ | rpoC2 | 0 | 0 | 0 | 0.00166 | 0 | 0 |
| 3   | A0A4Y5PPR0 | ~ | rpoC1 | 0.00065 | 0 | 0 | 0 | 0.00027 | 0 |
| 4   | A0A0M3TGK1 | ~ | rpoC1 | 0.00065 | 0 | 0 | 0 | 0.00027 | 0 |
| 5   | D3WDG9 | ~ | rpoC1 | 0.00065 | 0 | 0 | 0 | 0.00027 | 0 |
| 6   | T1Q0S2 | Maturase K (Fragment) (T1Q0S2_9ROSI) | matK | 0.00784 | 0 | 0.00784 | 0.00392 | 0 | 0.00784 |
| 7   | A0A126x286 | Putative LOV domain-containing protein (A0A126x286_LARTR) | N/A | 0.00036 | 0 | 0 | 0 | 0 | 0 |
| 8   | A0A140F7J3 | ~ | N/A | 0.00032 | 0 | 0 | 0 | 0 | 0 |
| 9   | A0A140F7J1 | ~ | N/A | 0.00041 | 0 | 0 | 0 | 0 | 0 |
| 10  | A0A0F6RA63 | Ribulose bisphosphate carboxylase large chain (Fragment) (A0A0F6RA63_9ROSI) | rbcL | 0 | 0 | 0.00015 | 0 | 0 | 0.00011 |
| 11  | Q0ZQ55 | ~ | ~ | 0 | 0 | 0.00016 | 0.00011 | 0 | 0 |
| 12  | Q9BAD9 | ~ | ~ | 0 | 0 | 0.00016 | 0.00011 | 0 | 0 |
| 13  | A0A2Z4N431 | ~ | ~ | 0 | 0 | 0 | 0.00012 | 0 | 0 |
| 14  | O20271 | ~ | ~ | 0 | 0 | 0.00016 | 0.00011 | 0 | 0.00012 |
| 15  | B3FD25 | ~ | ~ | 0 | 0 | 0 | 0.00011 | 0 | 0.00012 |
| 16  | B3FD36 | ~ | ~ | 0 | 0 | 0.00016 | 0.00011 | 0 | 0 |
| 17  | O20400 | ~ | ~ | 0 | 0 | 0 | 0 | 0 | 0.00012 |
| 18  | Q9TI23 | ~ | ~ | 0 | 0 | 0 | 0.00011 | 0 | 0 |
| 19  | A0A0F6TN85 | ~ | ~ | 0 | 0 | 0.00015 | 0 | 0 | 0 |
| 20  | A0A291L1Z1 | ~ | ~ | 0 | 0 | 0 | 0 | 0 | 0.00013 |
| 21  | Q9TI22 | ~ | ~ | 0 | 0 | 0 | 0.00012 | 0 | 0 |
| 22  | A0A3G1ZJ92 | ~ | ~ | 0 | 0 | 0.00016 | 0 | 0 | 0.00012 |
| 23  | B3FD32 | ~ | ~ | 0 | 0 | 0.00016 | 0.00010 | 0 | 0.00012 |
| 24  | A0A2U8T527 | ~ | N/A | 0 | 0 | 0.00015 | 0.00012 | 0 | 0 |
| 25  | A9XUT7 | ~ | rbcL | 0 | 0 | 0.00015 | 0 | 0 | 0 |
| 26  | A0A0F6TMP4 | ~ | ~ | 0 | 0 | 0 | 0.00011 | 0 | 0.00012 |
| No. | Accession (Uni Prot KB) | Description | Gene | NSAF (Abundance) |
|-----|------------------------|-------------|------|------------------|
|     |                        |             |      | C4 | T4 | C24 | T24 | C48 | T48 |
| 28  | B3FD26                 | ~           | ~    | 0  | 0  | 0   | 0   | 0   | 0   |
| 29  | A0A3G3NI82             | ~           | ~    | 0  | 0  | 0   | 0   | 0   | 0   |
| 30  | A0A0F6RA65             | ~           | ~    | 0  | 0  | 0.00015 | 0.00011 | 0   | 0.00011 |
| 31  | Q9TI21                 | ~           | ~    | 0  | 0  | 0   | 0   | 0   | 0.00012 |
| 32  | O20257                 | ~           | ~    | 0  | 0  | 0.00016 | 0   | 0   | 0   |
| 33  | A0A172C083             | ~           | ~    | 0  | 0  | 0   | 0.00034 | 0   | 0   |
| 34  | A0A142LSY1             | ~           | ~    | 0  | 0  | 0   | 0.00036 | 0   | 0   |
| 35  | O20289                 | ~           | ~    | 0  | 0  | 0   | 0.00012 | 0   | 0.00012 |
| 36  | O20239                 | ~           | ~    | 0  | 0  | 0   | 0.00012 | 0   | 0   |
| 37  | A0A1U9YH05             | ~           | ~    | 0  | 0  | 0   | 0.00039 | 0   | 0   |
| 38  | A0A3G3NI75             | ~           | ~    | 0  | 0  | 0   | 0   | 0   | 0.00012 |
| 39  | Q9THY9                 | ~           | ~    | 0  | 0  | 0   | 0   | 0   | 0.00011 |
| 40  | B3FD28                 | ~           | ~    | 0  | 0  | 0   | 0   | 0   | 0.00013 |
| 41  | Q9BAD8                 | ~           | ~    | 0  | 0  | 0   | 0   | 0   | 0.00012 |
| 42  | A0A3G3NI71             | ~           | ~    | 0  | 0  | 0   | 0   | 0   | 0.00012 |
| 43  | A0A4Y5PPU8             | ~           | ~    | 0  | 0  | 0.00015 | 0.00010 | 0   | 0.00011 |
| 44  | A0A3G4R1V6             | ~           | ~    | 0  | 0  | 0   | 0.00010 | 0   | 0.00012 |
| 45  | A0A4Y5PQN6             | ~           | ~    | 0  | 0  | 0.00015 | 0   | 0   | 0   |
| 46  | A0A0M3TGJ0             | ~           | ~    | 0  | 0  | 0   | 0.00010 | 0   | 0   |
| 47  | Q9SBY1                 | ~           | N/A  | 0  | 0  | 0.00016 | 0.00011 | 0   | 0.00012 |
| 48  | Q9SC24                 | ~           | ~    | 0  | 0  | 0   | 0.00011 | 0   | 0.00012 |
| 49  | Q9SC93                 | ~           | ~    | 0  | 0  | 0.00016 | 0.00011 | 0   | 0.00012 |
| 50  | Q9SBW8                 | ~           | ~    | 0  | 0  | 0   | 0   | 0   | 0.00012 |
| 51  | Q9SC53                 | ~           | ~    | 0  | 0  | 0.00016 | 0.00011 | 0   | 0   |
| 52  | Q9SBY0                 | ~           | ~    | 0  | 0  | 0.00016 | 0   | 0   | 0.000115 |
| 53  | Q9SBW6                 | ~           | ~    | 0  | 0  | 0.00016 | 0   | 0   | 0   |
| 54  | Q9SBX9                 | ~           | ~    | 0  | 0  | 0   | 0   | 0   | 0.00012 |
| 55  | Q9SBW7                 | ~           | ~    | 0  | 0  | 0   | 0   | 0   | 0.00012 |
| 56  | A0A1S6WN95             | ~           | ~    | 0  | 0  | 0   | 0.00054 | 0   | 0   |
The proteome analysis indicated that 19 Ribosomal proteins (RP) were differentially expressed in *T. propinqua* under different exposure time of heat stress including 9 h, but was upregulated after 48 h of heat stress in *T. propinqua* seedlings. These proteins included *rpl2*, *rpl36*, *rps8*, *rpl2*, *rpl12*, and *rps20*. The downregulated gene include *HS70* and *HS17.3*, *DOT-1* and *MatK* related to drought stress. The proteome analysis also revealed that 10 transcriptional genes differentially expressed in *T. propinqua* seedlings. Some genes are downregulated at heat stress for 4 h and 24 h such as *rpb2* and *PHYC*, but others are upregulated at heat stress for 48 h under heat stress as *EMB2765* and *PHYC*. Four proteins of *rpoC1* gene downregulated under heat stress with different exposure times; however, one protein of the gene *rpoC2* was upregulated in *T. propinqua* seedlings after 24 h of heat stress. These proteins identified DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four-ribonucleoside triphosphates as substrates in archaea. The Hsps with molecular weight varied (82–90 KD) classified to the Hsp90 family. In *Arabidopsis*, AtHsp90s are fundamental for tolerance to biotic and abiotic stresses. The small heat shock proteins (sHsps) with molecular weights varied (15–42 KD) may bind to partly folded or denatured proteins, that prevents irreversible unfolding or incorrect protein aggregation, or binds to unfolded proteins and permits additional refolding by Hsp70/Hsp100 complexes.

Plants also respond to heat stress through developmental, physiological, and biochemical ways, and these responses require expression of stress-responsive genes, which are regulated by a network of transcription factors (TFs), including heat stress transcription factors (HSFs) [30]. The transcriptional regulatory network and post-translational regulation of the transcription factors involved in the heat stress response [31]. The reactive oxygen species (ROS)-scavenging enzymes and HSPs are major functional proteins that are induced by heat stress and are well-known target genes of HS-responsive TFs [32, 33]. The results of this study revealed that 10 transcriptional genes differentially expressed in *T. propinqua* seedlings. Some genes are downregulated at heat stress for 4 h and 24 h such as *rpb2* and *PHYC*, but others are upregulated at heat stress for 48 h under heat stress as *EMB2765* and *PHYC*. Four proteins of *rpoC1* gene downregulated under heat stress with different exposure times; however, one protein of the gene *rpoC2* was upregulated in *T. propinqua* seedlings after 24 h of heat stress. These proteins identified DNA-dependent RNA polymerase catalyzes the transcription of DNA into RNA using the four-ribonucleoside triphosphates as substrates in archaea. However, tolerance to environmental stress during germination and seedling establishment may be supported by *rpoC* gene. Similar response patterns were reported, for example, *rpo* have different activities in tissues and developmental stages and may lead to variation in response to stress [35], in order to its expression discovered in the green and non-green tissues [36]. The over-expression of a chrysanthemum gene encoding an RNA polymerase II CTD phosphatase-like 1 enzyme enhances tolerance to heat stress [37]. The transcriptional response to heat stress in *Arabidopsis*, depend on the integrity of the RNA-dependent DNA methylation pathway [38]. In *Populus tomentosa* Carrunder heat stress upregulated and downregulated of various transcription factors, most downregulated genes were related to the light-harvesting complexes and photosynthetic electron transport system and the upregulated expression of some transcription factors at 12 h of heat stress [39]. Various genes in *Benincasa hispida Cogn.* var. induced after 4 days of heat stress [40].

Maturases are known to assist protein splicing factors in group II introns of most plants [41]. Transcriptional profile of some genes downregulated under drought stress such as proteins in roots of *Arachis duranensis* were identified as Cht2, MLP-34, heat shock proteins (HS70 and HS17.3), DOT-1 and MatK related to drought stress [42] and in *Populus cathayana*, an intron splicing related protein and MatK decreased under drought stress [43]. Under long drought stress in *Brassica napus* multiple organelle RNA editing factor 9, involved in RNA editing in mitochondria and plastids firstly increased and then decreased [44]. The changes of the transcription regulation of RNA processing-related proteins suggest that the variation and complexity of these proteins control the plants’ ability to overcome drought stress [45]. In the current study, one protein of the matK genes was downregulated for 4 h and 24 h but was upregulated after 48 h of heat stress in *T. propinqua* seedlings.

The proteome analysis indicated that 19 Ribosomal proteins (RP) translational genes were differentially expressed in *T. propinqua* under different exposure time of heat stress including 9 rpl and 10 rps genes. The downregulated gene include rpl20, rpl2, rpl36, rps8,
proteins of unknown gene were downregulated under heat stress of system regulating, photosynthetic capability, and stress defense gene expression. Developmental processes increased under sulphate deprivation, indicating that miRNAs may be induced by environmental conditions and not only by Arabidopsis 24 h and 48 h, and maize embryos for seedling establishment and post germination growth. Downregulated at 4 h of stress then upregulated at 48 h of stress. On the other hand, downregulated like upregulated such as in our study, 15 genes were differentially expressed in NDH tabacum. Incubation of ROS production performs a complex and highly dynamic process under undesirable growth conditions to organize a novel homeostasis. The highest downregulated at 24 h of heat stress; however, in barley, the expression of Rubisco large subunit under water deficit condition decreased, whereas rice has 57 RPS and 123 RPL genes copies. In rice, the rpl gene upregulated under abiotic and biotic stress [48], the differential expression of all the representative genes of rice performed under limited water and drought conditions at progressive time intervals [49].

Plants respond to stresses by a coordinate chloroplast and nuclear gene expression. In T. propinqua seedlings, the basic aim of regulated proteins and metabolites linked with photosynthetic pathways are chloroplast, thylakoid membrane, and nucleus [50]. Some genes were downregulated at 4 h like psbE, psaC, psbH, but others upregulated such as ycf4 under heat stress. Genes of psbE, ycf4, psaC and psbD are downregulated, but psbH and RCA2 upregulated at 24 h of heat stress. Other genes were downregulated at 48 h of heat stress as ycf3, psbK, psaB and psbH, but ycf4 and psaA were upregulated. In T. propinqua seedlings, the protein expression was downregulated in 20 protein of the rbcl gene after 24 h of heat stress compared to control. However, 16 proteins were upregulated in of rbcl gene after 24 h of heat stress. After 48 h of heat stress, the protein expression was upregulated in 27 protein of rbcl gene. This is in agreement with differentially expressed 25 photosynthesis-related proteins in two soybean varieties affecting RuBisCO regulation, electron transport, Calvin cycle, and carbon fixation during drought and heat stress [51]. In the drought tolerant cultivar of wheat, two proteins were down-regulated and three proteins of Rubisco-related proteins were not expressed [52]. In both wheat and barley, the expression of Rubisco large subunit under water deficit condition decreased [53, 54]. In the thylakoid membrane of rice, Rubisco plays a role in defense and regulation of photosynthesis under moderate heat stress [55]. In barley mutant, the defective P5S1 assembly causes photoinhibition and degradation of PSII due to a temperature-sensitive defect in ycf3 splicing [56]. The expression Ycf3 gene was downregulated after 4 h of heat stress in T. propinqua seedlings.

In T. propinqua seedlings, 16 differentially expressed proteins are involved in metabolic process. Genes of atpF and ndhJ were downregulated at 24 h of heat stress; however, ndhD and ndhI were upregulated at 24, 48 h of heat stress compared with the control. The ndhB gene was upregulated at 4 h of heat stress then was downregulated at 24 h of heat stress compared to control. Plant performs a complex and highly dynamic process under undesirable growth conditions to organize a novel homeostasis. The highest rates of ADP and NADP + produced in Arabidopsis thaliana under stress conditions, to protect photosynthetic activity by decreasing ROS production [57]. In light reaction, ATP and NADPH generated to use in the Calvin–Benson and the photorespiration cycles. Incubation of Hordeum vulgare under photooxidative conditions showed the largest increase in ndhA, because NDH could be involved in chloroplasts defense against photooxidative stress [58]. NDH activity and NdhK in chloroplasts increased in Nicotiana tabacum after exposure to heat stress at 50°C in the light [59]. In tobacco mutants, ndhB decreased under moisture stress, because NDH could retard the inhibition of photosynthesis [60].

In our study, 15 genes were differentially expressed in T. propinqua seedlings involved in transport processes. Some genes are upregulated such as ccmA at 4 h and 48 h of stress, AKT1, CAAT1, ccmB and clpP at 48 h of heat stress, but other genes were downregulated like DGAT1a, ccsA and VP1-2 at 24 h of heat stress. The DGAT1b was upregulated at 4 h of stress, but CAAT2 was downregulated at 4 h of stress when upregulated at 48 h of stress. On the other hand, VP1-1 was downregulated at 24 h of heat stress then upregulated at 48 h of heat stress with compared control. The AKT1 is pivotal in Arabidopsis under low-potassium conditions for seedling establishment and post germination growth [61]. CcmF messenger reacted through three-day-long cold exposure in maize embryos [62]. The upregulation of DGAT1 was under freezing stress in Boechera stricta lines might provide a common mechanism to allow freezing stress tolerance [63]. The structural/functional homolog of ABI3 in maize, indicate that VP1 and ABI3 play roles in the stabilization and activation of regulatory complexes involved in the transcription of target genes [64].

In this study, 4 genes were identified in T. propinqua seedlings with unknown function. Among them, the APS1 was upregulated at 24 h and 48 h, and PPS1 upregulated at 24 h, however, psbA was downregulated at 48 h of heat stress compared with the control. In Arabidopsis, miRNAs target superoxide dismutases, laccases, and ATP sulfurylases (APS) genes [65], one particular miRNA (miR395) increased under sulphate deprivation, indicating that miRNAs may be induced by environmental conditions and not only by developmental processes [65]. In tobacco, the Maize psbA gene was upregulated under drought stress tolerance out of antioxidant system regulating, photosynthetic capability, and stress defense gene expression [66]. Three putative LOV domain-containing proteins of unknown gene were downregulated under heat stress of T. propinqua seedlings. It controls the phosphorylation of an
amino acid residue in a protein. Protein kinases play a role in cellular processes, containing division, proliferation, apoptosis and differentiation [67]. The essential role of protein Tyr phosphorylation was in various plant processes and mostly in the regulation of developmental, abiotic and biotic stress responses [68]. Pais [69] suggested that phosphatases are unimportant enzymes that stop the activation of signaling cascades by kinases, Ser/Thr phosphatases type 2A (PP2A) performs positive and dynamic functions in stress signaling. In wheat, phosphor proteome analysis detected two serine threonine-protein kinases related to drought stress [70]. Under all abiotic stresses, protein tyrosine kinases PTK genes upregulated in rice [71].

In this study, one actin protein of unknown gene was upregulated after 4 h of heat stress in *T. propinqua* seedlings. This opinion agrees with different reports that actin is considered a fragment of 70-kDa heat shock to adapt to heat stress [72]. Experiments with heat sensing mutant *Arabidopsis thaliana* lines resulted in identification of an actin related protein 6 (ARP6) gene which plays a role in heat shock response [73]. In tobacco BY-2 cultured cells, heat shock stimulate the breakdown of actin microfilaments and modification in endoplasmic reticulum (ER) morphology connected by accumulation of the HSP70 binding protein (BIP). The analyzed cell structures (actin cytoskeleton and endoplasmic reticulum (ER) were reduced at (35 °C, 45 °C), but enhanced at 50 °C in the responses to heat stress [74].

**Conclusions**

Short exposure to heat stress alters the proteome profile in *T. propinqua* seedlings. Differential expression of 81 heat stress-responsive genes suggests that various signaling transduction pathways and molecular processes are affected due to heat stress. Differentially expressed 10 transcriptional and 19 translational genes are involved in heat shock protein (HSP), heat shock transcription factors (HSF) and chaperones associated increasing heat tolerance. Differentially expressed 14 gene are involved in photorespiration, photosynthesis, carbon fixation, reductive pentose-phosphate cycle, oxidation-reduction process and 16 genes are differentially expressed in metabolic process by increasing ADP and NADP+ rates under heat stress to protect photosynthetic activity by decreasing ROS production. In addition, 15 genes are differentially expressed in transport processes and 4 genes are identified with unknown function, such as the regulation of antioxidant system, photosynthetic capability, and stress defense gene expression. Two unknown genes are represented by putative LOV domain-containing Actin proteins, which may be involved in many plant processes and majorly found in the regulation of developmental, abiotic and biotic stress responses. These results are helpful to understand the plant's adaptation to heat stress.

**Methods**

**Samples preparation**

Seeds of *T. propinqua* ssp. *migahidii* were collected from May to July 2018 from plants growing in different sites in central of Saudi Arabia (Fig. 1). Permissions were not necessary for collecting these samples, as they did not distribute in nature reserves and also this species has not been included in the list of national key protected plants. Seeds collection habitats from several separate plants were randomly distributed throughout the whole region, used for various germination experiments. Germination of *T. propinqua* ssp. *migahidii* in response to heat was investigated by placing 50 seeds in a 9-cm-diameter Petri dish containing two layers of Whatman blotting paper No. 1, moistened with 5 ml of distilled water and 3 ml of distilled water added every 3 days' interval. The Petri dishes incubated at 25 and 40°C. The seedlings collected at 4, 24 and 48h of germination. The experiment replicated three times.

**Extraction of Protein**

Protein was extracted from seedlings. Samples materials (50 seedlings) homogenized in cold 100 mM phosphate buffer pH 7.8 containing 0.1 EDTA. The homogenates were centrifuged at 12000 rpm for 20 minutes to remove plant debris. Supernatants were used for total soluble proteins SDS-PAGE profile and proteomics analysis.

**Protein SDS PAGE Profile of Soluble Proteins**

Protein extract samples were mixed with sample loading buffer (Tris-HCl (0.5M pH 6.8), EDTA (0.25 M) SDS (5%), glycerol (10%), 2mg bromophenol blue, (7.15M) b-mercaptoethanol, ddH2O up to 10 ml) at equal volume and 10 µl samples were loaded onto a vertical slab gel in a Bio-Rad apparatus according to the method of Laemmli [24]. The run buffer was added to the upper tank just before running. This gel was run at 80 volts for a quarter of an hour, then the voltage was raised to 120 volts until the samples reach
one inch from the bottom of the gel. The gel was removed from the apparatus gently and placed in a plastic tank, and covered with fixing solution of 30% ethanol, 10% acetic acid overnight, followed by rinsing briefly in 5% acetic acid, then treated with sensitizer working solution of 25µL composed of 12.5 mL Sensitizer and 12.5 mL water and stained using “Pierce Silver Stain Kit” (Catalog number: 24612), using silver stain enhancer (0.125mL of enhancer and 12.5mL stain). Developer working solution (0.125mL of enhancer and 12.5mL developer). The 10% SDS gel was washed with ultrapure water for 5 minutes twice. Then, fixing solution was added to the gel for 15 minutes at room temperature. This step was repeated twice followed by gel washing with the 10% ethanol for 5 minutes twice. The gel was washed again with ultrapure water for 5 minutes twice, incubated in Sensitizer working solution for exactly 1 minute, and then washed twice with ultrapure water for 1 minute. Silver stain enhancer was added for 5 minutes followed by gel wash with ultrapure water for 20 seconds. Developer working solution was added until protein bands appear in 2-3 minutes. When the desired band intensity was reached, developer working solution was replaced with stopping solution. The gel was washed briefly, and then replaced by incubation in acetic acid for 10 minutes. The protein ladder used as a molecular weights marker (MW) ranged between 10 - 250 KD “Precision Plus Protein™ Unstained Protein Standards” (Catalog number: 1610396) according to manufacturer instructions. The stained gel was photographed while wet.

Protein Sample Preparation for Proteomics

Protein extraction from gel and denaturation

Sixty µl of lysis buffer containing 8M Urea, 500mM Tris pH 8.5 and protease inhibitor (1:100) was added to the samples. Then samples were shaken vigorously and centrifuged at 10,000 rpm for 30 minutes at 4ºC, followed by BCA protein quantification using “Pierce Detergent Compatible Bradford Assay Kit” (Catalog number: 23246) according to manufacturer instructions.

Protein digestion

Each protein sample was reduced using 2µl of 200 mM DTT (Dithiothreitol) with shaking using vortex, and spin down and incubated for 45 min at room temperature, and then alkylated using 2µl of 1M IAA (lodoacetamide) and incubated at room temperature for 45 in the dark followed by 102 µl of 100mM Tris pH 8.5. Trypsin was added to digest the samples at 6 µl trypsin containing 1ug procaine enzyme and incubated overnight at 37ºC with shaking at 900 rpm then the sample was acidified to pH 2-3 by adding 6ul of 100% formic acid, followed by spin down for 30 min at room temperature.

The Phosphorylated Peptides

By using the stage tip “Pierce™ C18 Spin Tips” (Catalog number: 84850) the peptides were activated by adding 15ul methanol on the tip, the initialization was washed by using 15ul from “solution B” (0.2% FA+ 80% ACN). The re-equilibration was washed twice using 15ul from “solution A” (0.2% FA) and the samples were added to new Eppendorf tube, and then washed twice with 15µl “solution A”, respectively. Then, the phosphorylated peptides were eluted with elution buffer “solution B” in a collection tube 3 times followed by speed-vac followed by re-constituting the samples in 22 µl of “solution B”. In this stage, the samples were centrifuged between each step at 3000 RPM. Inject the samples on Mass spectrometry.

The peptides quantification

Peptides were quantified by measuring the concentration by using the bicinchoninic acid assay (BCA assay), incubated at 95ºC for 5 min, followed by adding 1000 µl from prepared BCA followed by incubating at 60ºC for 30 min, and then cooled down at room temperature for 20 min. The samples were measured at A 562.

Chromatography

The LC system using a Nano-LC system consisted of Eksigent nano-LC 400 autosampler attached with Ekspert nano-LC425 pump. Chromatographic separation of samples was performed on a trapping cartridge CHROMXP C18CL 5um (10x0.5 mm) pumped at flow rate of 10 ul/min for 3 min using mobile phase A. Chrom-XP C18CL column (3 um, 120A, 150 x 0.3mm) at a flow rate of 5 ul/min. The injection volume of all samples was 5 µl. Trap and elute needle wash was done two cycles using 10% isopropanol in injection mechanism with analysis time of 55 minutes. Mobile phases were MillIQ containing 0.1% FA (A) and acetonitrile containing 0.1 %FA (B). The following gradient profile of mobile phase was used: 0.0 min, 97% A, 3% B; 38 min, 70% A, 30% B; 43 min, 60% A, 40% B; 45 min, 20% A, 80% B; 48 min, 20% A, 80% B; 49 min, 97% A, 3% B; 57 min, 97% A, 3% B.
Mass spectrometry

The LC-QTOF system analysis was carried out using a Sciex TripleTOF™ 5600+ in a positive acquisition mode with TOF MS survey scan followed by production scan for the most abundant 40 ions (High-resolution mode). The source conditions were as follows: cycle time is 1.5 sec; TOF mass range, 400 – 1250 m/z; MS2 range (product ion), 170 – 1500 m/z; ion selection threshold, 150 cps; Total run time, 55 min and MS calibration, Sciex tuning solution (P/N 4457953). All MS parameters controlled by Analyst TF 1.7.1(Sciex software) for data acquisition. Raw MS files from the TripleTOFTM 5600+ is converted into MGF files then analyzed by Peptide shaker (v1.16.43). Database used is Uniprot *Zygophyllum* organism (swiss-prot containing 931 proteins).

The search parameters

The measured parameters were as follows: Trypsin digestion, identification algorithms X!Tandem. Max missed cleavages 2. Precursor m/z tolerance 20.0 ppm. Fragment m/z tolerance 10.0 ppm. Precursor charge 2-5 isotopes 0-1. modification: 1- fixed modification; carbamidomethylation of C (Mass; 57.02). 2- variable modification; acetylation of K (Mass; 42.01), acetylation of protein N-term (Mass; 42.01), deamidation of N (Mass; 0.98), deamidation of Q (Mass; 0.98), oxidation of M (Mass; 15.99).

Statistical analysis

Each experiment was performed in three replicates and statistical analysis was made using Excel software and a principal component scatter diagram constructed by using the software PAST Version 3.22. The PCA applied to assign the variables to genes and proteins based on their tolerance to heat stress.

Abbreviations

*T. propinqua*: *Tetraena propinqua*, SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis, ROS: reactive oxygen species, HSPs: heat shock proteins, TFs: transcription factors, HRPs: heat stress-responsive proteins, LC-MS: liquid chromatography–mass spectrometry, UniProt: a freely accessible database of protein sequence and functional information, many entries being derived from genome sequencing projects, EDTA: Ethylenediaminetetraacetic acid, Tris-HCl: Trizma® hydrochloride, SDS: sodium dodecyl sulfate, ACN: acetonitrile, FA: Formic acid, LC-QTOF-MS/MS: Qualitative tandem liquid chromatography quadrupole time of flight mass spectrometry, MGF: mascot generic format files, BCA assay: bicinchoninic acid assay, GO: gene ontology, PCA: principal component analysis.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this published article. Database used is Uniprot (https://www.uniprot.org) *Zygophyllum* organism.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MEA-E; conceptualization, methodology, writing-review and editing, formal analysis, investigation, project administration, HAA; resources, funding acquisition, supervision. All authors have read and approved the manuscript.

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Figure 1

Representative image of SDS-PAGE gel, indicating little changes in protein profile among control and treated plants under heat stress. Control (C) and treated (T) for 4, 24 and 48h under 25oC and 40oC, respectively.

Figure 2

Histogram illustrating the functional classification of identified and unidentified genes from T. propinqua seedlings categorized by their major function based on information from Uni-Prot databases and gene ontology.
**Figure 3**

Diagram illustrating the percentage of the major functional proteins in cellular components.

**Figure 4**

The PCA-Biplot of the 81 gene differentially expressed in T. propinqua seedlings under heat stress. Control (C) and treated (T) for 4, 24 and 48h of exposure under normal conditions (25oC) and heat stress (40oC), respectively.
Figure 5

PCA-Biplot for the 59 top differentially expressed proteins in T. propinqua seedlings under heat stress. Control (C) and treated (T) for 4, 24 and 48h of exposure under normal conditions (25°C) and heat stress (40°C), respectively.

Supplementary Files

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- Pictureforthegel.jpg