The response of *Hymenocrater longiflorus* to water stress and proline application under in vitro cultivation

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Abstract

Hymenocrater longiflorus Benth. (surahalala) is a wild plant species having properties to be categorized as both pharmaceutical and ornamental plants. To date, the genomics of this plant is unknown and the gene expression profiling of the genes related to its metabolite has never been studied before. In order to study the responses of surahalala plant grown under *in vitro* conditions to abiotic stresses and the deferential expressions of the genes related to its essential oils under exogenous proline application; three levels of PEG6000 (0, 10, and 20%) and five levels of proline (0, 5, 10, 15, and 20 μ M) were applied in mixture with its culture medium. Accordingly, induced water deficit increased oxidant levels while decreased fresh weight of surahalala tissues; whereas, application of proline up to 15 μ M was able to relatively compensate the negative effect of water deficit. Contrarily, high proline level (20 μ M) showed negative effect surahalala plants which is probably due to the stress simulation (nutrition) in this plant under high proline concentration. Since PEG and proline applications increased essential oil contents, the best combination of proline and PEG treatment in the surahalala plant for achieving the highest content of its essential oils were 10 μ M and 10% levels, respectively. The expression profiles of TPS27, L3H, TPS2, TPS1, OMT and GDH3 in this plant were successfully carried out and the results verified the involvement of these genes in 1,8-cineole, carvone, α -pinene, thymol, estragole, and β -Citronellol biosynthesis, respectively. In addition, our results indicated that these genes could get involved in the other metabolite synthesis under water deficit conditions.

1. Introduction

Tissue culture is a successful method of producing plants independent of the environments which has helped researchers to increase the performance of their studies. In addition, this technique is very applicable in growing plants having difficulties in their production through seeds such as *Hymenocrater longiflorus* Benth. This plant is native to the northwest of Iran (Kurdistan province) and northeast of Iraq (Kurdistan state) and it has been known as surahalala by natives. Surahalala could be categorized as both pharmaceutical and ornamental plants (Ahmadi et al., 2010). However, it has never been grown under field or greenhouse conditions by farmers and it is just available in high altitude places of its origins as a result of its seed low viability and difficult seed germination. Therefore, there are rare studies on this plant and most of them have been carried out by the institute of the authors. In a previous study (Hoseiny et al., 2021), the authors have described how to be successful in growing this plant under *in vitro* condition. According to traditional pharmaceutical plant texts and traditional pharmacists that use plants to cure the illnesses along with a roughly recent study (Ahmadi et al., 2010), surahalala is able to reveal different pharmaceutical properties such as anti-allergic, anti-inflammatory, and sedative effects. Related to some biochemical properties of surahalala, Shahriari et al. (2013) have reported a high antioxidant activity (e.g., SOD and POD) and secondary metabolite (e.g., carvacrol and thymol) contents in its shoots. Hoseiny et al. (2021) showed the content of that essential oils in the shoots of this plant are properly enough to be used for pharmaceutical and essential oils extraction uses.

According to Hoseiny et al. (2021), using any extra biochemical compounds along with the standard substances in the culture media for generating and growing surahalala almost certainly can stimulate or suppress the production of some essential oils in this plant. Since the quality and quantity of the essential oils that is produced by this plant is an important feature in its pharmaceutical potential, study the application of different compounds and substances on the altering its essential oil content is necessary (Ni et al., 2021). To date, to the best of our knowledge, the only study that has studied the impacts of extra substances on the contents of the essential oil of surahalala was carried out by Hoseiny et al. (2021). They have studied the impacts of applying salicylic acid and simvastatin (SV) in mix with the medium and showed that both of these substances are able to highly change the content of essential oils in surahalala. On the flip side, there are many references available indicating the crucial influence of environmental stresses on essential oils and metabolite content of different plants (Abdelmajeed et al., 2013). Langroudi et al. (2013) showed that drought stress can change essential oils contents of *Rosmarinus officinalis* and affect the quantity of its final production. Saed-Moucheshi et al. (2021) stated that drought stress is able to increase the antioxidant contents of triticale as a result of the higher expression rate of related genes.

Proline is an essential compound in plants, mainly under stress condition (Shakeri et al., 2019). Proline, as a free amino acid in plant cells and tissues, is mostly involved in osmotic adjustment and antioxidant activities under different stresses (Saed-Moucheshi et al., 2017). In addition, pharmaceutical properties and application in brewing industries have been reported for the free amino acid. Saed-Moucheshi et al. (2014) reported antioxidant activities for proline in different plant species. Different reports have verified the impacts of proline as an enhancer or suppressor different gene expression (Nounjan et al., 2012). This substance has upregulated the expression of some abscisic acid (ABA) such ABA1, ABI1 and AXR2 gene in *Arabidopsis* which are mainly involved in stress-responsive pathways (Cao et al., 2020). There are also other reports such as Li et al. (2018), Lee et al. (2019), (Poorghadir et al., 2020), and Sofy et al. (2020) related to the interaction of proline with jasmonic and salicylic acid gene networks. Although the studies on proline application on essential oils under water deficit conditions are rare, some studies (Poorghadir et al., 2020, Werrie et al., 2020) showed that proline may be able to indirectly affect the molecular pathways of some metabolites.

Despite of the fact that surahalala has a great potential to be used in pharmaceutical industries, there are rare studies on the effect of different treatment on its phytochemical contents. In addition, this plant has never been studied under drought stress condition or proline treatment. Regarding the ability of drought stress and proline treatment in altering the quantity and quality of plants' productions and essential oils contents, as well as unavailability of any studies related to these treatments on surahalala, this study aimed to consider the effects of PEG application, as drought stress simulator, and proline on quantity and quality of some essential oils and phyto-biochemical content of surahalala. Furthermore, the expression profiling of six different genes involved in biogenesis pathways of some essential oils. Also, any possible associations of the expression rate with biochemical contents of surahalala have been considered by different methods of data mining.

2. Materials And Methods

2.1. Plant materials and treatments

Plant samples of surahalala have been collected with the help of knowledgeable natives from its natural habitats in Kurdistan, Iran, with 35.2526 °N and 46.2612 °E geographic coordinates and 1435 m altitude in 2020. These samples were selected from the youngest, healthy, and the full-grown herbaceous shoot parts of the plants and they carefully transformed to the Biotechnology and Tissue culture lab of Horticultural Science, University of Kurdistan. After preparing the samples through washing and sanitizing they were cultured in MS (Murashige & Skoog, 1962) medium prepared based on the instruction that was formerly introduced by the mentioned lab and is fully described in Hoseiny et al. (2021). In summary, the prepared medium was containing all necessary substances such as sucrose, vitamins, and myo-inositol, as well as 5 mg/L benzyl aminopurine (BAP) (Sigma Aldridge Company Ltd.) and 0.1 mg/L indole butyric acid (IBA) (Merck KGaA Company). Due to the presence of PEG6000 as a treatment in the current study, agar-containing media could not be solidified, so synthetic cotton was used instead of agar. After that, the previously produced explants in Biotechnology and Tissue culture lab (~ 3.5 cm in length) were transferred to glass jars (250 mL) containing the medium. Following that, both PEG and proline treatments were mixed with the prepared media. Three levels of PEG containing 0, 10, and 20% V/W were applied to simulate the water shortage condition along with five levels of proline consisting of 0, 5, 10, 15, and 20 µM. Each treatment was applied in one glass jar containing 4 explants and was repeated 8 times (3 PEG, 5 Pro, 8 rep; 125 overall units). The glass jars were placed in a growth chamber Convivon, CMP 4030, Canada) under ultraviolet (UV) light ($46 \mu\text{Em}^{-2}\text{s}^{-1}$ light intensity) the light produced by fluorescent light instrument with 16/8 h illumination/darkness conditions along with $25 \pm 1^\circ\text{C}$ temperature and $55 \pm 2\%$ relative humidity. After 56 days from starting the experiment, when plants were fully expanded, the sampling of the measurements was done.

It should be noted that the plant material that was used for this experiment is now available in Tissue Culture Lab, Horticultural Science Dept., University of Kurdistan and they are reproduced and grown under the *in vitro* condition. Therefore, if the researchers and scientists want to carry out any experiment on this plant, the authors can provide the plant materials. In addition, the authors claim that all the materials that were used in this study comply with relevant institutional, national, and international guidelines and legislation. The *Hymenocrater longiflorus* species is produced and grown naturally and some part of west of Iran and east of Iraq, but this plant is not in danger of distinction because it is naturally reproduced in these parts annually. However, we comply with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. Furthermore, the key for distinguishing this species and a dried plant specimen is available in the herbarium of Horticultural Science Dept., University of Kurdistan and Dr. A.A. Mozafari is the one to identifying this plant and gathered and provided the specimen according to international laws and policy.

2.2. Feature measurements and essential oil components

After finalizing the experiment, the whole plants (two cub-samples) were taken out and the root and shoot were separated from the media and were weighed using an accurate digital scale (AND Japan FX400; 0.00001 g accuracy) after removing the medium by washing with double distilled water. Shoot fresh weight (SFW) and root fresh weight (RFW) were recorded for each unit and were reported as the average of the two sub-samples in each glass jar according to gram unit.

After weighing the shoot samples, 0.5 g of these samples were used for measuring free proline content (Bates et al., 1973), hydrogen peroxide (H₂O₂) content (Beers & Sizer, 1952), and superoxide dismutase activity (SOD) (Dhindsa et al., 1981) by using using a spectrophotometer (UV-2100 model SUV, New Jersey) method.

Ten grams of the shoot samples were used for extracting the contents of the essential oil in three randomly selected glass jars (repeat). The hydro-distillation method was applied for extracting essential oil components via using the Clevenger method. The complete method of extraction is fully described in Hoseiny et al. (2021).

2.3. Expression rate and RExpressin package development

In this study, a package in R language has been developed which is able to extract the direct results of gene expression from the Ct (threshold cycle) output of both favorite and reference genes in real-time PCR. This package named 'RExpressin' its source as well as its instruction use is available online in a GitHub repository (<https://github.com/ArminSaed/RExpression>). Also, a copy of this package, one in 'zip' format for Windows users and one in 'gz' format for Linux users is presented as supplementary material in this paper. The mentioned package was installed on RStudio and used for calculating the expression rate of the considered genes in this study involving essential oils and secondary metabolite production pathways. The method of delta Ct was used for preparing the 'RExpression' and is described in summary as follows.

Determined Ct of the favorite gene was subtracted from the Ct of reference gene as ΔCt . After that, ΔCt of each treatment level (here 3 PEG levels by 5 proline levels) was subtract from the average ΔCt of the control treatment (no PEG or proline for the current study). Finally, by assuming the complete performance of PCR, the $2^{-\Delta\Delta Ct}$ formula was used for relative expression rate where the rate of the control is equal to 1. In the developed package, the column number of treatment variable and reference gene should be specified. The out of this package is containing the average expression rate of each treatment as well as its SE. This package is also able to draw 10 different plots for comparing the treatments and also to test the quality of the results which is specified by a number from 1 to 10.

2.4. Quantitative RT-PCR

The total RNA of *Hymenocrater longiflorus* shoots of all treated units were extracted by using RNA extraction kit (Cinapure, Cinagene, Iran) based on the manufacturer's protocol. The quantity of RNA was considered by spectrophotometer method (Biochrom, United Kingdom). The direct electrophoresis (Bioanalyzer, Agilent, USA) of total RNA using agarose gel was applied to determine the quality of the

total RNA in which the 16s and 24s rRNA were distinguishable. Total RNA was then transcribed into cDNA using the Reverse Transcription Kit (Cinapure, Cinagene, Iran) according to the instruction of the manufacturer. In order to survey the quality of cDNA, the acting primers were used for PCR and the product was transformed to agarose gel where two bands were sharply observable.

The RT-PCR was performed using StepOnePlus Real-Time PCR System containing 96 wells (Applied Biosystems StepOnePlus™ system, USA). Three biological replicates per treatment were considered for RT-PCR. Two internal control genes (Actin and elongation factor; EF1) were applied as internal controls. The relative expression levels of TPS27 gene may also call 1,8-cineole synthase1 (involved in 1,8-cineole synthesis), Limonene-3-hydroxylase (L3H) gene (involved in carvone synthesis), pinene synthase (TPS2) gene (involved in α -pinene synthesis through MVA pathway), TPS1 gene (involved in thymol synthesis), OMT or O-methyltransferases gene or (involved in estragole synthesis) and GDH1 (Geraniol dehydrogenase) gene (involved in β -Citronellol synthesis). Due to the lack of genomic data of *surahalala*, we considered all sequences of the mentioned genes in its closet species and after finding the similar motif by running the blast test on NCBI site, the primers were mostly designed through these similar sequences by using Allele ID software. Before using these primers in final RT-PCR efficiency and quality were checked by changing the temperatures of PCR stages and adding or removing one or some of the nucleus based mostly from the 5' end (Supplementary Table S1).

2.5. Statistical analysis

Factorial experiment based on a completely randomized design was applied in this experiment with PEG and proline as the first and second experimental factors, respectively. The data were subjected to analysis of variance (ANOVA) and mean comparison based on the least significant method (LSD) with $p = 0.05$ using package agricolae in RStudio 1.4.1 software (R core v. 4.0.5). After obtaining the significant letters based on the mean comparison, the standard errors of the mean (SE) were then calculated for each treatment. Correlation plot according to the Pearson linear method with 0.05 significant probability was carried out by 'ggcorrplot' library. In addition, heatmap according to the Euclidian distance Ward method (Saed-Moucheshi et al., 2013) using 'pheatmap' and 'gplot2' library, principal component analysis (PCA) and its and biplot representation based on paired correlation by 'factoextra' library, and canonical correlation analysis via 'CCA' library were performed.

3. Results

3.1. Essential oils, biochemical and growth traits

Table 1 shows multiple mean comparisons for examined features containing essential oil phytochemical contents and biochemical traits. Under all levels of PEG, the higher concentration of proline up to 15 μ M decreased SOD activity, while application 20 μ M increased its activity. On the other side, the higher PEG concentration dramatically increased the activity of SOD. The highest SOD activity was recorded for the highest water shortage level (20% PEG) and no application of proline. Under 10% PEG, proline showed its highest contents in comparison with other PEG levels. Under no water shortage condition, application of

10 μM proline caused the plant to increase the content of proline. However, the content of free proline in the surahalala showed an increasing pattern in response to the higher concentration of proline under both moderate (10% PEG) and severe (20% PEG). Mean comparison of H_2O_2 indicated that the higher the content of PEG application in the medium, the higher the content of H_2O_2 in surahalala plants. On the contrary, increasing the content of proline lead to lower content of H_2O_2 , except in 20 μM proline level that showed H_2O_2 content almost equal to no proline application. Under all water shortage levels, from control to 15 μM applications of proline increased the SFW, while higher proline content decreased SFW. Quite the contrary, the severe water shortage conditions lower growth of surahalala plants and lower SFW. RFW showed no specific pattern response to application of proline under no PEG application, but under 10% PEG it showed a growing pattern in response to proline levels. Application of 10% PEG, induced the highest RFW in surahalala, while 20% PEG application significantly decreased the root growth compared with 10% PEG. The RFW under both 10% and 20% PEG application was recorded for 15 μM proline application.

Table 1
Mean comparison of PEG by proline interaction levels for the content of essential oils and other measured features in surahalala plants.

PEG	Proline	SOD	PRO	H ₂ O ₂	SFW	RFW	
0%	0 µm	2 ± 0.095h	0.25 ± 0.005h	8.97 ± 0.209ij	16.3 ± 0.38c	1.6 ± 0.032de	
	5 µm	2.6 ± 0.026f	0.55 ± 0.014f	8.18 ± 0.228ij	18.76 ± 0.144b	1.93 ± 0.046b	
	10 µm	1.45 ± 0.019i	0.71 ± 0.013c	8.2 ± 0.137ij	21.41 ± 0.135a	1.4 ± 0.016f	
	15 µm	1.96 ± 0.033h	0.49 ± 0.011f	7.92 ± 0.136j	18.32 ± 0.438b	1.47 ± 0.039ef	
	20 µm	2.9 ± 0.031e	0.42 ± 0.008g	9.21 ± 0.37hi	9.96 ± 0.136d	1.92 ± 0.034b	
10%	0 µm	3.55 ± 0.035d	0.7 ± 0.013cd	13.65 ± 0.307e	6.19 ± 0.149f	2.32 ± 0.027a	
	5 µm	2.46 ± 0.055fg	0.41 ± 0.014g	12.56 ± 0.153ef	5.81 ± 0.5f	2.3 ± 0.025a	
	10 µm	2.35 ± 0.027fg	0.91 ± 0.018a	11.43 ± 0.191fg	7.93 ± 0.185e	1.76 ± 0.035c	
	15 µm	2.29 ± 0.03g	0.85 ± 0.017ab	10.39 ± 0.295gh	10.47 ± 0.103d	1.68 ± 0.028cd	
	20 µm	3.34 ± 0.035d	0.9 ± 0.01a	19.7 ± 0.132b	6.99 ± 0.427ef	0.63 ± 0.029j	
20%	0 µm	7.11 ± 0.033a	0.63 ± 0.017e	22.23 ± 0.173a	5.79 ± 0.244f	0.8 ± 0.046i	
	5 µm	6.31 ± 0.04b	0.63 ± 0.017de	18.67 ± 0.311bcd	4.4 ± 0.232gh	1.33 ± 0.02fg	
	10 µm	4.13 ± 0.039c	0.87 ± 0.008ab	18.39 ± 0.324cd	6.4 ± 0.119f	1.24 ± 0.038g	
	15 µm	2.98 ± 0.045e	0.83 ± 0.018b	17.72 ± 0.15d	5.12 ± 0.256fg	1 ± 0.013h	
	20 µm	6.44 ± 0.086b	0.63 ± 0.013e	19.45 ± 0.328bc	3.25 ± 0.076h	0.71 ± 0.014ij	
PEG	Proline	1,8-Cineole	Carvone	α-Pinene	Thymol	Estragole	β-Citronellol
	0 µm	0.12 ± 0.002h	0.13 ± 0.002i	0.09 ± 0.002g	0.08 ± 0.001h	0.7 ± 0.016ef	0.18 ± 0.004i

PEG	Proline	SOD	PRO	H ₂ O ₂	SFW	RFW	
0%	5 μm	0.59 ± 0.02f	0.43 ± 0.025h	0.09 ± 0.002g	1.09 ± 0.033g	0.39 ± 0.018g	0.17 ± 0.02i
	10 μm	0.64 ± 0.011ef	2.63 ± 0.024b	0.09 ± 0.004g	3.02 ± 0.016d	0.24 ± 0.067h	0.3 ± 0.013i
	15 μm	0.84 ± 0.01d	2.33 ± 0.006c	0.03 ± 0.002i	4.16 ± 0.018c	0 ± 0.009j	0.5 ± 0.003h
	20 μm	0.11 ± 0.002h	0.09 ± 0.02ij	0.1 ± 0.002fg	0.78 ± 0.054g	0.01 ± 0.021j	0.3 ± 0.032i
10%	0 μm	0.96 ± 0.011cd	1.87 ± 0.009d	0.11 ± 0.004ef	1.98 ± 0.014e	0.79 ± 0.027de	0.99 ± 0.011f
	5 μm	1.21 ± 0.007b	1.46 ± 0.035e	0.09 ± 0.002g	3.21 ± 0.05d	0.9 ± 0.006cd	1.6 ± 0.006d
	10 μm	1.15 ± 0.019bc	2.74 ± 0.037a	0.08 ± 0.002h	4.13 ± 0.069c	0.61 ± 0.014f	2.73 ± 0.055b
	15 μm	3.25 ± 0.018a	1.52 ± 0.008e	0.12 ± 0.003e	9.22 ± 0.055a	0.44 ± 0.023g	4.17 ± 0.029a
	20 μm	0.02 ± 0.014i	0 ± 0.031j	0.11 ± 0.001ef	7.1 ± 0.069b	0.06 ± 0.002ij	2 ± 0.01c
	0 μm	0.69 ± 0.054e	1.8 ± 0.02d	0.18 ± 0.002b	0.96 ± 0.154g	2.85 ± 0.01a	0.67 ± 0.083g
	5 μm	0.45 ± 0.008g	0.67 ± 0.001fg	0.19 ± 0.003a	0.83 ± 0.028g	1.16 ± 0.003b	0.57 ± 0.033gh
	20%	10 μm	1.07 ± 0.002c	0.57 ± 0.001g	0.16 ± 0.002c	3.29 ± 0.013d	0.97 ± 0.002c
20%	15 μm	0.5 ± 0.003g	0.01 ± 0.002j	0.14 ± 0.002d	1.66 ± 0.118f	0.15 ± 0.001hi	1.67 ± 0.04d
	20 μm	0.01 ± 0.001i	0.68 ± 0.009f	0.17 ± 0.003bc	0.8 ± 0.013g	0.67 ± 0.016f	0.83 ± 0.017f
Mean ± standard error of mean							
In each column, means with the same letter(s) are not significantly different (LSD 5%)							

Applying the data mining methods and multiple mean comparisons indicated that the contents of all six measured essential oils, generally, grew up in response to PEG application. The α-pinene and estragole showed their highest content under 20% application of PEG; however, the contents of 1,8-cineole, carvone, thymol, and β-citronellol were the highest under 10% application of PEG. Also, α-pinene and estragole showed reduction patterns in response to higher proline levels, while for other essential oils, 10 and 15 µM proline application brought about the highest contents. On the other hand, estragole and α-pinene

essential oil contents showed positive and significant correlation with each other while they had no significant correlation with 1,8-cineole, Thymol, and β -citronellol (Fig. 1A). 1,8-cineole, Thymol, and β -citronellol showed positive and significant correlations with one another and their highest correlation was between Thymol and β -citronellol (0.82). Carvone showed negative correlations with estragole and α -pinene and its correlation with other three essential oil content was not significant. Cluster analysis of the essential oils showed the similar pattern in which estragole and α -pinene were placed into one cluster, carvone was contained in one cluster and Thymol, β -citronellol, and 1,8-cineole were set into another separate cluster (Fig. 1B). Biplot showed that Carvone along with Thymol, β -citronellol, and 1,8-cineole were placed in the same quarter which contained 15%, 10%, and no application of proline under 10% PEG treatment (Fig. 1C).

Pearson correlation indicated that SOD, proline content, and H_2O_2 had positive significant correlations with one another while they presented negative correlations with root and shoot weight of surahalala (Fig. 1A). Additionally, SFW and RFW were grouped in the cluster of carvone (Fig. 1B). The cluster of estragole and α -pinene contained SOD and H_2O_2 content. According to cluster analysis of treatments (Fig. 1D) and the biplot, except 20 μ M proline, all other levels of proline treatments under no PEG application were grouped together with high similarity. Application of 20 μ M proline under no PEG application was contained in the cluster of 10% PEG application.

3.2. Expression profiling

Expression profiling of six genes involved in sensual oils and secondary components in surahalala plant grown under *in vitro* condition was assessed and their expression levels were standardized according to two different reference genes, EF1 and actin, which their mean comparison in each treatment level is represented in Supplementary Table S2 and Table S3. Since the correlation between the results related to both reference genes was high (over 0.85), their mean profiling has used as final expression levels and the mean comparisons of the treatments regarding all the six genes are provided in Table 2. The expression of 1,8-cineole, Carvone, Alph_P, and β -citronellol genes in 10% PEG application were higher than other control (no PEG) and 20% PEG; however, 20% PEG application showed an expression level for Thymol and estragole (Table 2). Application of different levels of proline under 20% and no PEG application showed caused some small changes in expression profiling of the considered genes. On the flip side, these genes have strongly responded to the proline application under 10% PEG application and their expression went through many changes. Under 10% PEG treatment, 10 and 15 μ M proline caused a higher expression rate than other proline levels. Figure 2 shows the expression rate of all considered genes versus the treatment levels. Generally, the expression level of 1,8-cineole and β -citronellol genes were higher than other genes, particularly under 10 and 15 μ M proline levels along with 10 and 20% PEG applications.

Table 2
Mean comparison of PEG by proline interaction levels for the expression rate of the selected genes in surahalala plants.

PEG	Proline	TPS27	L3H	TPS2	TPS1	OMT	GDH1
0	0	1 ± 0f	1 ± 0f	1 ± 0b	1 ± 0i	1 ± 0hi	1 ± 0gh
0	5	0.84 ± 0.012f	0.78 ± 0.011fg	0.27 ± 0.006fg	1.02 ± 0.014i	0.8 ± 0.013hi	2.14 ± 0.05fg
0	10	0.39 ± 0.004f	0.77 ± 0.008fg	0.08 ± 0.001i	1.54 ± 0.015h	0.1 ± 0.001i	6.53 ± 0.09e
0	15	3.5 ± 0.053e	2.38 ± 0.025e	0.2 ± 0.003h	2.67 ± 0.034g	0.36 ± 0.004i	6.25 ± 0.13e
0	20	2.27 ± 0.069ef	1.88 ± 0.034e	0.31 ± 0.005ef	1.02 ± 0.017i	3.76 ± 0.071f	1.78 ± 0.03gh
10	0	6.4 ± 0.085d	4.81 ± 0.049d	2.07 ± 0.028a	3.63 ± 0.038f	6.45 ± 0.064e	3.59 ± 0.059f
10	5	4.16 ± 0.089de	2.11 ± 0.041e	0.28 ± 0.004fg	1.66 ± 0.019h	2.95 ± 0.054fg	3.54 ± 0.047f
10	10	30.39 ± 0.583b	18.22 ± 0.344a	0.54 ± 0.013d	10.98 ± 0.203c	8.13 ± 0.168d	46.49 ± 1.211a
10	15	94.39 ± 1.73a	13.38 ± 0.203b	0.35 ± 0.005e	11.63 ± 0.12b	5.48 ± 0.116e	27.51 ± 0.329b
10	20	21.48 ± 0.427c	10.64 ± 0.209c	0.68 ± 0.018c	3.56 ± 0.067f	34.88 ± 0.707b	6.08 ± 0.181e
20	0	0.1 ± 0.002f	0.01 ± 0.001h	0.11 ± 0.001i	0.42 ± 0.005j	2.24 ± 0.034fgh	0.16 ± 0.002h
20	5	0.1 ± 0.003f	0.01 ± 0.002h	0.09 ± 0.001i	1.76 ± 0.017h	2.27 ± 0.036fgh	1.42 ± 0.02gh
20	10	0.42 ± 0.006f	0.25 ± 0.003gh	0.24 ± 0.003gh	9.87 ± 0.108d	28.87 ± 0.514c	15.94 ± 0.202c
20	15	0.13 ± 0.001f	0.07 ± 0.001h	0.11 ± 0.003i	14.21 ± 0.247a	1.92 ± 0.021gh	12.57 ± 0.354d
20	20	0.15 ± 0.003f	0.17 ± 0.002h	0.53 ± 0.006d	8.5 ± 0.083e	62.68 ± 0.797a	5.56 ± 0.073e
Mean ± standard error							
In each column, means with the same letter(s) are not significantly different (LSD 5%)							

Correlation coefficient analysis indicated a positive and significant association of Carvone, β-citronellol, and 1,8-cineole with one another (Fig. 3A). Accordingly, clustering of the considered genes placed Carvone, β-citronellol, and 1,8-cineole into one cluster, α-pinene and estragole in a separate cluster, and

Thymol in another cluster (Fig. 3B). These results were clearly verified by biplot where genes in the same cluster were placed near each other (Fig. 3C). According to the clustering results of treatments (Fig. 3D), all treatment levels were incorporated with three separate clusters where 10 and 15 μ M under 10% PEG treatment were closely placed in the same cluster. Also, the clustering results of the treatments were corroborated by biplot results.

Cross-correlation between expression of the considered genes versus the biochemical and growth-related features are presented in Fig. 4A. Accordingly, the expression rate of 1,8-cineole gene had the highest correlation (0.84) with its essential oil component, 1,8-cineole contents. The expression level of α -pinene was negatively correlated with α -pinene content. In order to summarize these cross-associations, the canonical correlation for expression profiling as one set and other measured features as another set was carried out. The results showed that the first four canonical coefficients (CC) out of six showed a significant explanation of cross-relationship of the two sets (Supplementary Table S4). The members of the two sets were portrayed as a 2D-plot based on the first and second CC (CC1 and CC2) in which proline content showed close proximity with expression rate of Thymol, β -citronellol, Carvone, and estragole along with the content of β -citronellol and H_2O_2 (Fig. 4B). The activity of superoxide dismutase was placed near the contents of Carvone, α -pinene, and 1,8-cineole essential oils. Among the expression profiling of the six genes, α -pinene expression rate was closest to shoot and root weights of surahalala.

4. Discussion

Up to the best of our knowledge, there is no study on the effect of environmental stresses on surahalala plants. However, according to previous studies (Çakmakçı et al., 2020, Morshedloo et al., 2017, Pirbalouti et al., 2014), essential oil contents and the expression profiling of their related genes along with the growth of aromatic plants are significantly affected by altering the environmental conditions. Chrysargyris et al. (2020) reported that water deficit is one of the major environmental factors changing the synthesis and production of secondary metabolites in medicinal plants. Moreover, such stress condition can cause lower plant growth and matter produced in significant amounts. On the other hand, proline has reported being an effective free amino acid in response to environmental stresses where it can play as osmoregulatory elements in plants. In most cases application of exogenous proline led to higher tolerance in plants under water deficit stress. However, its application has showed different effects on the contents of secondary metabolites and the expression levels of their related genes (Cui et al., 2021, Ganguly et al., 2020, Hussain et al., 2021, Li et al., 2014, Silva et al., 2018, Zouari et al., 2019). In order to quantify the effect of PEG application as a simulator of water deficit condition and the effect of proline in such situations, SOD activity and H_2O_2 content were measured. The results, according to higher H_2O_2 content and SOD activity under higher PEG levels showed that the PEG application was able to properly simulate the stress condition. In addition, the content of free proline increased in response to PEG application in surahalala plants. Though, PEG led to a significant decrease in plant growth by affecting the shoot and root weights. Moreover, the results of proline application on SOD activity and H_2O_2 content, on one hand, and on the fresh shoot and root weights of the surahalala plants, on the other hand,

indicated that higher concentration of proline than 15 μM might causes a stress-like condition in this plant. The application of proline up to 15 μM showed mainly a decrease in the SOD activity and the content of H_2O_2 along with higher shoot and root weights in comparison with the control, under all PEG levels. The content of proline, however, was continuously increased in response to a higher level of exogenous application of proline. These results were verified by different advanced data mining methods in which the associations of SOD and H_2O_2 with root and shoot weights were highly negative. In most of the previous studies the applications of proline resulted in higher dry matter production and the tolerance of the plants (Zali & Ehsanzadeh, 2018). Although, our study verified that higher contents of proline than a critical point might lead to a negative effect on the plants. In concordance with our study, different authors showed that proline regulation application was able to decrease the negative effects of environmental stress on *Trifolium repens* L. (Li et al., 2014), tobacco (Silva et al., 2018), *Glycyrrhiza uralensis* (Cui et al., 2021), and chickpea (Hussain et al., 2021) mostly via decreasing the content of H_2O_2 and malondialdehyde and also increasing the activity of enzymatic antioxidants. Also, Zali and Ehsanzadeh (2018) and (Aggarwal et al., 2011) showed that proline was capable of increasing the growth of fennel And *Phaseolus vulgaris* under water deficit conditions and nutrient deficiency.

Measuring secondary metabolites in the current study indicated that the PEG application has a positive effect on the contents of most essential oils. On the flip side, in all secondary metabolites such as 1,8-cineole, Carvone, Thymol, and β -citronellol the highest PEG level (20%) did not show the highest content, instead, it showed lower contents compared with the 10% level of PEG. This result indicates that environmental stresses might play a role in activating some genes that are involved in both essential oils synthesis pathways and stress-responsive genes. Nonetheless, the complex polyploid levels in line with the lack of genomic and transcriptomic information related to the majority of aromatic plants have limited further study even on model plants such as mint species (Chen et al., 2021). In some studies (Alonso-Ramírez et al., 2009, Emamverdian et al., 2020, Pirbalouti et al., 2014), associations between genes that are involved in ABA, jasmonic acid and gibberellin responsive pathways which are enhanced under specific stresses and the metabolite gene network has been reported.

In line with our results, Akula and Ravishankar (2011) and Aftab (2019) reported higher secondary metabolites in response to environmental stresses, but there is no study related to the stress effect on secondary metabolite contents of surahalala. Meanwhile, there were positive associations between 1,8-cineole, Carvone, Thymol, and β -citronellol contents with proline content in surahalala and they showed low Euclidian distances from one another as well as placing in similar clusters. These essential oils were increased by increasing the level of proline up to 15 μM and then decreased dramatically. The α -pinene and estragole contents showed a negative response to proline application. Since 1,8-cineole, carvone, thymol, and β -citronellol levels were increased in response to 10% PEG and up to 15 μM proline in surahalala plants, it seems that low water deficit condition and moderate application of proline may a proper combination of treatment leading to increase their concentrations per dry matter unit in this plant. On the other hand, α -pinene and estragole showed negative associations with proline, but their contents were generally equal to, or in some cases higher than, the control proline treatment. Therefore, the

suggested treatment combination of low water deficit level and moderate application of proline would not have significant negative impacts on these compounds.

As a class of secondary metabolites, monoterpenes are mainly identified in fungi, but different contents of them have also been reported in some medicinal plants such as eucalyptus (Shaw et al., 2015) pepper, mint, and recently in surahalala (Hoseiny et al., 2021). These compounds showed variety of functions in plants and are involved in some basic and specialized metabolisms. Some of these compounds play a role in odor synthesizing for attracting pollinators. There are reports related to their operations in defense mechanisms against herbivores and plant pathogens by synthesizing some toxic compounds. There are some studies indicating that these compounds are involved in signal transduction under various stresses after the starting damage to the plant cell (Hoseiny et al., 2021). In the current study, H_2O_2 as a signaling molecule showed significant and positive associations with α -pinene and estragole based on the results of data mining, which are concordance the previous studies (Yauk et al., 2015, Zali & Ehsanzadeh, 2018). Other reports regarding the importance of monoterpenes are related to their impacts on the symbiosis relationship of their plants by accumulating in their roots and rhizomes (Melo et al., 2020, Tarraf et al., 2015). In addition to the importance of monoterpenes on the plants, different effects of these compounds on human diseases have been also reported.

In aromatic and pharmaceutical plants, the content of essential oils, and terpenoid compounds are important substances with high economic values. The major contents of surahalala essential oils, according to Mozafari *et al.* (2021), are consisted of monoterpenes. 1,8-cineole is a commercially significant monoterpene compound that has pharmaceutical applications and is considered as a potential biofuel. According to the literature, this compound has anti-inflammatory impacts and Linghu et al. (2019) reported that it was able to subside the inflammatory effect of human umbilical vein endothelial cells (HUVECs) disease phenotype (Vosoughi et al., 2020). Similarly, carvone is known to have anticarcinogenic properties in human tissues. Carvone is also able to synthesis attractive odors and flavors applicable in aromatherapy and food products (Bouwmeester et al., 1998). In our study, carvone content showed high Euclidian distances from SOD activity and H_2O_2 indicating revers associations between these compounds in some conditions. In line with these results, Huchelmann et al. (2014) described that carvone is able to hinder the production of stress-induced compounds and secondary metabolites in tabacum leaf. In addition to 1,8-cineole, pinene has anti-inflammatory and also antimicrobial activities. Surahalala plants under the water deficit and proline treatments showed to have thymol in their shoot, which is able to be used as an effective antimicrobial compound. Having antiseptic effects caused thymol to be used as an ingredient in mouthwash and toothpaste products (Yauk et al., 2015). Another monoterpene that was distinguished in surahalala plants is estragole, which can act as an agent for attracting the pollinators, play a role in defense mechanisms against pathogens and herbivores, and be applied in food products and spices as flavoring compounds (Ebadollahi, 2020). Similarly, β -citronellol is used in perfume factories and can act in pollinators attractions along with repellent effects on some organisms, mainly mosquitoes (Xie et al., 2018).

As verified by the results of this study and mentioned in the results of some other studies (Langroudi et al., 2013, Nounjan et al., 2012, Poorghadir et al., 2020, Werrie et al., 2020), different the environmental conditions lead to change in the contents of secondary metabolites. The contents and biosynthesis of the monoterpenes are normally regulated by similar or different molecular and genomic pathways which are most commonly functioning as the connected gene networks. Altering environmental conditions or treating plants with various compounds may directly trigger or repress some genes in monoterpenes genomic pathways, or the genes that are responsible for transcribing their sequences into RNAs. The levels of changes under altered conditions could be quantified by expression rates of related genes and considering the transcriptomic patterns of the plant tissues. In this study, all considered monoterpenes were affected by the stress condition and proline treatments. Hence, assessing the expression rates of the genes involved in their biosynthesis can help us to find the origin of the changes in such metabolites and lead the way to future genetic engineering toward upregulation or downregulation of their expression. The monoterpenes that are considered in this study are the product of enzymatic activities transforming specific precursors.

According to Chen et al. (2021), terpene synthase (TPS) gene family is largely involved in terpenes biosynthesis of *Mentha longifolia*. In their study, the TPS family was divided into six subfamilies sharing a great number of mostly identical and some similar motifs. After considering these motifs in all available databases of the aromatic plants we found over 90 percent similarity among these motifs indicating that these subfamilies are most likely available in surahalala with similar sequences and tasks. However, sequence analyses of complete cds of different TPSs within and between different species by Huchelmann et al. (2014) indicated that the similarity of these genes within each species genome is significantly higher than the similarity between genomes of different species.

Almost all of the precursors of different monoterpenes are products of similar pathways in which TPS genes act at near the end of these pathways. In these pathways, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are synthesized at the initial points. IPP and DMAPP are direct products of two other pathways consist of the mevalonate pathway (MVA) in the cytosol and the phosphate pathway (MEP) in the chloroplast. Generally, IPP and DMAPP are directly transferred into geranylgeranyl pyrophosphate (GGPP), farnesyl diphosphate (FPP), and geranyl diphosphate (GPP) by catalyzing activities of prenyltransferases. After that, different TPS genes catalyze these compounds into the precursors of monoterpenes and other secondary metabolites. The produced precursors are then modified by enzymatic activities transcribed from different enzyme genes (Chen et al., 2021).

The content of 1,8-cineole in this study was higher than its content in other plants, and the proline treatments, especially under water deficit conditions sufficiently increased the levels of this monoterpene. Huchelmann et al. (2014) attributed the low level of 1,8-Cineole in *nicotiana tabacum* to rapid conversion of GPP precursor into other monoterpenes other than 1,8-Cineole. Therefore, proline treatment under water deficit condition most likely trigger the expression of some genes involved in either higher production of 1,8-Cineole precursor or more rapid conversion of the precursor GPP into this monoterpene in surahalala plants. Other than treated surahalala plants, control plants showed slightly higher 1,8-

cineole content than other plants. In the study of Chen et al. (2004), 1,8-Cineole has shown negative effects on germination and significantly reduced its percent in *Arabidopsis*; consequently, the problems with direct germination of surahalala plants may be the results of higher 1,8-cineole content in this plant which led us to produce it under *in vitro* condition. On the other hand, under moderate water deficit and slight concentration of proline (5 μ M) the relative expression rate of TPS27 gene, which is involved in the production of 1,8-cineole precursor, was significantly higher than both control condition and high concentrations of PEG (20%) as well as proline (20 μ M).

The expression rate of L3H gene was significantly increased by applying PEG and proline treatments in surahalala which its highest rate was achieved by 10 or 15 μ M proline under 10% PEG. The expression rate of this gene showed a positive and significant correlation with carvone content in surahalala and based on the data mining results, the Euclidian distance of this monoterpene and L3H expression rate was significantly lower than other gene expression profiling. Carvone results from the MEP pathway that produces geranyl diphosphate (GDP) and it showed negative regulatory effects on the MVM pathway resulted in negative association and high distance between this monoterpene and other assessed monoterpenes in this study, for example, estragole, that are produced by MVM pathway. After biosynthesizing of GDP, limonene synthase (LS) enzyme converts it to limonene by separating the diphosphate group. Next, hydroxylation of limonene (C6 position) by L3H and L6H by co-factoring of NADPH transforms it into carveol and finally into carvone by dehydrogenases mechanism (MetaCyc, 2008). One of the reasons for increasing the content of carvone under water deficit conditions in the current study may be the production of signaling molecules such as O_2^- and OH^- that are capable of dehydrogenated different compounds. On the other hand, proline might get involved directly in the L3H production pathway or indirectly help NADPH cofactor in the carvone pathway, lead to higher expression profiling of L3H in both ways. In the study of Xie et al. (2018) higher gibberellin content, which is affected by stress conditions, showed positive interaction with carvone content.

The anti-inflammatory and antimicrobial of α -pinene have verified by different studies. According to Wu et al. (2021), α -pinene is a specific product from aromatic plants which most other organisms are not able to produce it. α -pinene is directly synthesized by the activity of the pinene synthase (PS) enzyme from GGPP. In the current study, the expression rate of TPS2 gene was increased in response to application 10% PEG, while it decreases by applying 20% PEG. This result indicates that moderate stress on surahalala plant can lead to higher conversion of GGPP into this monoterpene. The results of data mining verified these results and showed that the expression rate of TPS2 and also the content of α -pinene were positively correlated and they had a low Euclidian distance from those treatments that contained 10% PEG in a two-dimensional representation of canonical correlation. Under no stress and severe stress conditions expression rate of TPS2 showed no regular pattern in response to proline treatments, while its rate was the highest under moderate stress level (10%PEG) with no proline application. Therefore, proline treatment has a negative regulatory effect on the expression rate of TPS2. To the best of our knowledge, there is no study related to the effect of proline treatment on the expression rate of the TPS2 gene or the content of α -pinene. Wu et al. (2021) showed that in addition to the

enzymatic activity of TPS2, a fusion of the pinene synthase (PS) with heterologous geranyl diphosphate synthase (GPPS), they called GPPS-PS significantly increased the α -pinene and β -pinene content.

In the previous study by Hoseiny et al. (2021), thymol was detected in surahalala shoots. In the current study, the content of thymol was increased as the result of proline treatments and PEG application, however, the best combination for achieving higher thymol content was 15 μ M proline level applied under 10% PEG as moderate water deficit condition. One pathway of biosynthesis of thymol is through the conversion of GPP to neryl pyrophosphate (NPP) which in turn transforms to γ -terpinene and then into p-cymene. The final product of this pathway is either carvacrol or thymol. In surahalala both of these monoterpenes were detected by the gas chromatography method used in this study; though, the content of thymol was higher in almost all samples. Majdi et al. (2017) described TPS1 as a significant agent in thymol biosynthesis. The expression profiling of this gene (TPS1) showed similar pattern to the content of thymol in response to the treatments. The main product of TPS1 activity in the metabolite biosynthesis pathway is γ -terpinene, one of the precursors of thymol. Canonical correlation and correlation plot showed that TPS1 gene expression in addition to low two-dimensional distance and a high correlation with thymol, showed a close relationship with β -Citronellol content as well. Additionally, the relative expression of TPS1 showed significant association with 1,8-cineole content based on the results from heatmap analysis. This means that TPS1 is probably involved in other metabolite biosynthesis pathways in surahalala and it likely is not a pathway-specific enzyme.

The availability of estragole metabolite in surahalala was first reported by Hoseiny et al. (2021). The importance of this metabolite in surahalala is related to its role as a defense compound against different microorganisms and herbivores, in addition to pollinator attractants (Ebadollahi, 2020). Estragole is a subset of the phenylpropenes group that also contains isoeugenol, eugenol, and transanethole (isoestragole). A part of the flavoring properties of some plant species such as banana (*Musa sapientum*) (Jordán et al., 2001), melon (*Cucumis melo*) (Aubert & Pitrat, 2006), tomato (*Solanum lycopersicum*) (Ortiz-Serrano & Gil, 2010), and strawberry (*Fragaria vesca*) (Aragüez et al., 2013) is resulting from the phenylpropenes group available in these fruit in their sequestered glycosides or as free volatiles forms. Estragole is normally biosynthesized via IPP pathway which its precursor is the compounds of a para-hydroxy group such as conferyl acetate (Yauk et al., 2015) and p-allylphenol (Gross et al., 2002). O-methyltransferases (OMTs) is an important enzyme act in transforming para-hydroxy compounds to estragole by using a methyl donor (S-adenosylmethionine; SAM) (Ebadollahi, 2020). The content of estragole in surahalala plants was significantly increased in response to the higher water deficit condition and higher proline treatments. Similarly, the expression profiling of OMT genes showed positive response to these treatments. Unlike other considered metabolites in this study, the highest content of estragole in surahalala plants was obtained under the highest proline level (20 μ M) and the highest PEG concentration (20%). The effectiveness of these treatments come from their impact on methyl productions pathways because some methylated compounds could be used in the signal transduction activity in response to stress conditions. As it was mentioned earlier, the highest level of proline in surahalala plant is probably caused nutrient stress or make water deficit severer leading to activating some networks in stress signaling pathways that take advantage of methylated compounds.

β -Citronellol is another metabolite that was detected in surahalala and its availability in this plant was also reported previously by Hoseiny et al. (2021). This metabolite significantly increases the importance of using surahalala extracts in industrial products as the result of its application in insect (especially mosquito) repellents and perfumes (Dehsheikh et al., 2020). β -Citronellol content and the expression rate of geraniol dehydrogenase, GDH3, showed a significant and positive correlation (0.73) resulting from their similar patterns in response to PEG and proline applied in this study. Based on the clustering results of metabolites and the different expressions of considered genes in this study obtained from heatmap method, GDH3 expression rate and β -Citronellol showed the closest relationship with 10 and 15 μ M proline and 10% PEG treatments. GDH is one the significant enzymes act in the biosynthesis of graniol and citronellol by dehydrogenizes the GPP precursors. Moreover, the GDH3 expression rate showed low Euclidian distances in two-dimensional plots of biplot and canonical correlation from TPS1 and L3H expression rates and the thymol content in surahalala.

5. Conclusion

The results of this study clearly verified that *Hymenocrater longiflorus* as a hardly known plant species is capable of being used and developed as an important pharmaceutical plant. Considering the impacts of water stress by the PEG application and proline treatment showed that water deficit increases oxidant levels while decreases fresh weight of surahalala tissues; whereas, application of proline up to 15 μ M was able to relatively compensate the negative effect of water deficit. The results also indicated that a high proline level (over 20 μ M) can act stress simulator in surahalala plants and have a negative effect on its growth. In addition, the best combination of proline and PEG treatment in the surahalala plant for achieving higher content of its essential oils were 10 μ M and 10% levels, respectively. Even though the sequence of different genes in surahalala is unknown, we could saucerful assessed the expression profiling of TPS27, L3H, TPS2, TPS1, OMT, and GDH3 in this plant by considering the sequences of these genes in closely related plant species and distinguishing highly similar domains. These genes showed to be actively involved in 1,8-cineole, carvone, α -pinene, thymol, estragole, and β -Citronellol synthesis, respectively. In addition, our results indicated that these genes could get involved in the other metabolite synthesis under water deficit conditions. Additionally, an R package was developed in this study that is able to estimate the relative expression rate of any considered gene by taking its cycle threshold (Ct) point of internal and target gene.

Declarations

Conflict of interest

The authors claim to have no conflicts of interest to disclose.

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Figures

Figure 1

Correlation plot (A), heatmap (B), biplot (C), and clustering (D) of the essential oil contents and measured features in surahalala plants under PEG and Proline treatments. S0_p0: 0% PEG and 0μM proline; s0_p5: 0% PEG and μM proline; s0_p10: 0% PEG and 10 μM proline; s0_p15: 0% PEG and 15 μM proline; s0_p20: 0% PEG and 20 μM proline; s10_p0: 10% PEG and 0 μM proline; s10_p5: 10% PEG and 5 μM proline; s10_p10: 10% PEG and 10 μM proline; s10_p15: 10% PEG and 15 μM proline; s10_p20: 10% PEG and 20 μM proline; s20_p0: 20% PEG and 0 μM proline; s20_p10: 20% PEG and 10 μM proline; s20_p15: 20% PEG and 15 μM proline; s20_p20: 20% PEG and 20 μM proline; SOD: superoxide dismutase activity; PRO: proline content; H2O2: hydrogen peroxide; SFW: shoot fresh weight; RFW: root fresh weight.

Figure 2

Radar graph for comparing relative expression levels of the used genes under different treatment levels of PEG and proline in surahalala plants. S0_p0: 0% PEG and 0μm proline; s0_p5: 0% PEG and μm proline; s0_p10: 0% PEG and 10μm proline; s0_p15: 0% PEG and 15μm proline; s0_p20: 0% PEG and 20μm proline; s10_p0: 10% PEG and 0μm proline; s10_p5: 10% PEG and 5μm proline; s10_p10: 10% PEG and 10μm proline; s10_p15: 10% PEG and 15μm proline; s10_p20: 10% PEG and 20 μm proline; s20_p0: 20% PEG and 0μm proline; s20_p10: 20% PEG and 10μm proline; s20_p15: 20% PEG and 15μm proline; s20_p20: 20% PEG and 20μm proline.

Figure 3

Correlation plot (A), heatmap (B), biplot (C), and clustering (D) of the expression profiling of essential oils genes in surahalala plant under PEG and Proline treatments. S0_p0: 0% PEG and 0µm proline; s0_p5: 0% PEG and µm proline; s0_p10: 0% PEG and 10µm proline; s0_p15: 0% PEG and 15µm proline; s0_p20: 0% PEG and 20µm proline; s10_p0: 10% PEG and 0µm proline; s10_p5: 10% PEG and 5µm proline; s10_p10: 10% PEG and 10µm proline; s10_p15: 10% PEG and 15µm proline; s10_p20: 10% PEG and 20 µm proline; s20_p0: 20% PEG and 0µm proline; s20_p10: 20% PEG and 10µm proline; s20_p15: 20% PEG and 15µm proline; s20_p20: 20% PEG and 20µm proline.

Figure 4

Correlation plot (A) and canonical correlation biplot (B) for relative expression of the selected gene versus essential oils content and other measured features in surahalala plants. SOD: superoxide dismutase activity, PRO: proline content, H2O2: hydrogen peroxide, SFW: shoot fresh weight, RFW: root fresh weight.

Supplementary Files

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