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Original Article

Investigating the Key Genes Expression of Parthenolide Biosynthetic Pathway in Vegetative Stages of Feverfew Leaves under Different Levels of Drought Stress

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Abstract

Sesquiterpene lactones are the main categories of terpenoids in that are abundant in Asteraceae family, they are biologically valuable due to have a key role in the interaction with the environment. Feverfew (*Tanacetum parthenium*) of the Asteraceae family has been used as a refrigerant in the past and its most important constituent among the sesquiterpene lactones is parthenolide. Parthenolide that is Zhrmakranvlyd a germacranolide lactone, is very important due to the medical and pharmacological activity, in particular, factor preventing migraine and treatment of cancer. In the present study, leaves of feverfew (young and mature leaves) under drought stress at different levels were studied in order to study changes in the expression of key genes in parthenolide biosynthetic pathway includes germacrene A synthase (Tp GAS) and parthenolide synthase (Tp PTS) through Real Time PCR methods. The results showed that the expression of genes germacrene A synthase (Tp GAS) and parthenolide synthase (Tp PTS) showed significant difference in the leaves of plants under different treatments of stress than the control plants and the highest expression of germacrene A synthase and parthenolide synthase was occurred in treatment which plant was at severe drought stress (irrigation once every 7 days). The gene expression of studied genes was different in different stages of growth and reduction in gene expression was observed in mature leaves in compare to young leaves.

Key words: Parthenolide biosynthesis pathway, Drought stress, Tp PTS, Tp GAS

INTRODUCTION

Asteraceae family plants are the largest family of flowering plants, had been used in the past and even now as a medicinal plant. Feverfew is herbaceous plant belonging to the Asteraceae family lasting two or more years that having shoot straight with longitudinal grooves at height of 30 to 80 cm that its leaves are soft, green, long petioles, oval form and dividers blade. This plant is native to Kazakhstan, Central Asia and the Mediterranean region. It can be found as a wild plant in the northern province of Iran such as Golestan, Mazandaran, Gilan and Azerbaijan, Tehran, Hamedan and centeral province. *Tanacetum parthenium* was used as a traditional medicine, especially to reduce fever as well as insect repellant. This plant has pharmacological activity, for example anti-cancer, anti-inflammatory, and antispasmodic.

It is also used to treat fever and migraine headaches, abdominal pain, dental pain, infertility, menstrual problems and alleviate delivery pains. Medicinal effects of this plant in leaves and flowers can be attributed to the presence of sesquiterpene lactones and flavonoids. Sesquiterpene lactones are the main categories of terpenoids that are abundant in the Asteraceae family and are biologically remarkable. parthenolide is a germacranolide from Sesquiterpene lactones which is a major component of feverfew leaves. This compound has been recently received much attention due to the increasing Medicinal value and Pharmacology activities, as a migraine prophylactic and

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cancer treatment. I is responsible for biosynthesis of terpenes in plants at the path methyl erythritol phosphate (MEP) pathway in the plastid and mevalonic acid (MVA) in the cytosol that germacrene A synthase in the biosynthesis of castenolide (a common precursor of sesquiterpene lactones derived from germacronolid) plays a role in circulating farnesyl diphosphate (FDP) to germacrene A (Figure 1).

In this study, gene expression pattern was evaluated on two key gene of parthenolide biosynthesis (GAS) and (PTS) in the leaves of the vegetative growth stage (young and mature) under drought stress at four different levels (control, mild, moderate and severe).

MATERIALS AND METHODS

This study was conducted in order to investigate the effect of different levels of drought stress on the genes expression involved in the parthenolide biosynthesis of the feverfew plant (*Tanacetum parthenium*) in 2016 in the greenhouse of Islamic Azad University. This study was performed in a factorial experiment at completely randomized design with three replications.

Drought treatments included four levels (control, mild, moderate and severe). Feverfew seeds were prepared from Pakan-e-Isfahan Company and were transferred in plastic pots with a c of sandy culture bed of sand, peat, manure and field soil.

In the early vegetative stage, drought stress was imposed in four levels of without stress and mild stress (3 days irrigation with 400 ml water), mild stress (5 days irrigation) and severe (once irrigation every 7 days). After 30 days of drought stress, sampling was done from young leaves. Sampling of mature leaves was done in late of September and leaf samples were stored in a freezer -80°C. Real Time PCR reaction was performed using Step-one device from ABI Company (Figure 2).

Each stage of proliferation was conducted as follows: separation stage at 95°C for 15 seconds, 60°C for 60 seconds during. After completing cycles of PCR, melting curve at the temperature 0.3°C in each cycle and between the temperature 65-95°C to determine the specificity of PCR reactions.

Studied gene expression rate was calculated using the delta method. Accordingly, the average cycle threshold ΔCT (from two replication) for each sample was calculated, then the same action was carried out for House-Keeping genes and finally, the expression of candidate genes was obtained using the following formula:

Gene expression: $2^{-\Delta \text{ (House-Keeping Gene)-}\Delta CT \text{ (Target Gene)}}$

Table 1: Primers used for gene expression of different genes via real time PCR

Primers	Sequence	Tm
TpGAS-F	5- TGCTATCTCGGGTACTTTCAAGG-3	61.42
TpGAS-R	5-TTCTCCTCTTATTCTCAACTGTGCG-3	61.81
GAPDH-F	5-GTTGACTTGACTGTGAGACTTGAGC-3	61.49
GAPDH-R	5-CCTTGAGGTTGCCTTCGGATTC-3	64.86
TpPTS-F	5-AGACATTACGTTTACACCCTCCCG-3	63.09
TpPTS-R	5-ATCACGACACAAGTCCCAGGG-3	63.02

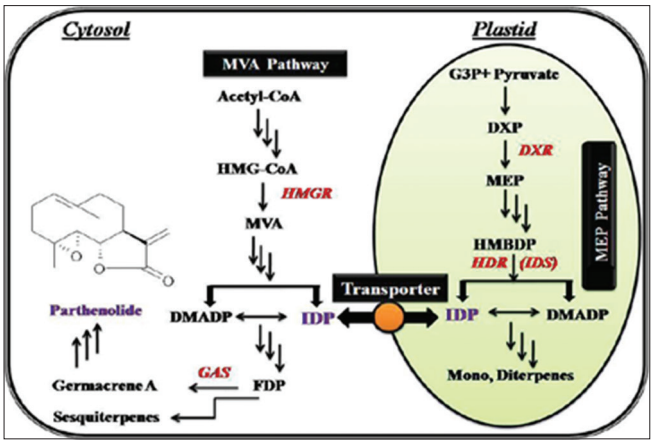

Figure 1: The biosynthetic pathway of parthenolide

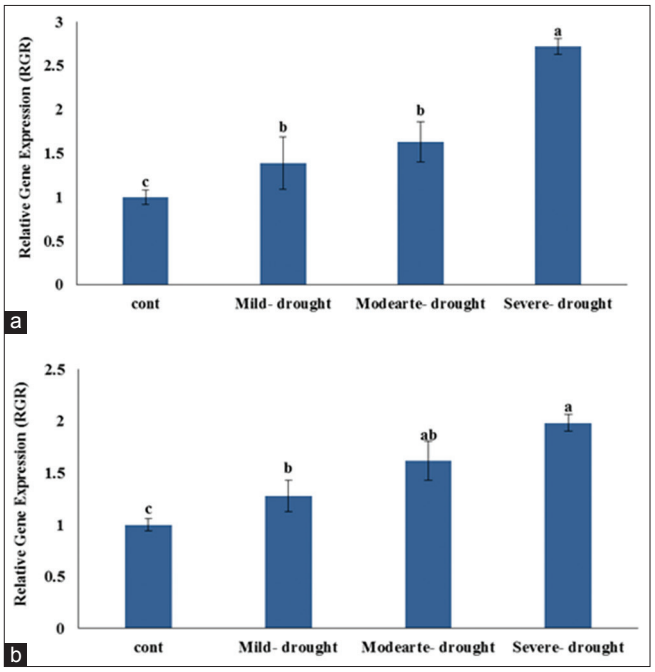

Figure 2: (a) The pattern of GAS gene expression in the biosynthesis pathway of parthenolide in young leaves of feverfew (The first stage of vegetative phase), (b) The pattern of PTS gene expression in the biosynthesis pathway of parthenolide in young leaves of feverfew (The first stage of vegetative phase)

RESULTS AND DISCUSSION

The results of variance analysis to assess the gene expression of parthenolide biosynthetic pathway (Tp GAS) and (Tp PTS) in different stages of growth (in young leaves and mature leaves of feverfew) showed that there was a significant difference at different treatments (different levels of drought) ($p \leq 0.01$). Germacrene A synthase gene expression (GAS Tp) and parthenolide synthase (Tp PTS) showed a significant increase in young leaves of plants under drought stress compared to control plants leaves ($p \leq 0.01$) (Figure 2). A significant increase was observed in mature leaves in expression of studied genes in compare to the leaves of control plants at severe drought level (once every 7 days irrigation). also increased gene expression was observed at the other different levels (mild and moderate) while this increase was not significant ($p \geq 0.05$) (Figure 3).

As can be seen in Figure 2 and 3, the highest gene expression was observed in compared to other treatments while the plant was under severe drought stress (irrigation once every 7 days)., Also, there was not significant differences in studied genes expression at the leaves under light drought stress (once irrigation every 3 days) and moderate (once irrigation every 5 days), but it was observed significant difference in plants under drought stress with control and other treatments. The active ingredients in the organs and plants are never constant and it can be changed proportional to the stages of plant growth and some environmental conditions. According to Pellati et al. (2005), the quantity and quality of phytochemicals depends on genetic diversity, environmental conditions and plant phenology. Environmental conditions of drought stress are different in plants and drought is one of the most important factors that limit plant growth and crop production. When the loss of water in the form of transpiration is higher than water absorption, water stress is occurred and the long-term stress affects all metabolic processes.

Pettopodos et al. (2008) stated that the drought can affect on some compounds and secondary metabolites in medicinal and aromatic plants. Holtzer et al. (1998) believe that the drought stress can have an effect on reducing or increasing of secondary metabolites levels. Terpenoids that are the largest group of secondary metabolites, there are abundant in feverfew, as more than 12% of sesquiterpene of feverfew is parthenolide and consider as the active ingredient of this plant which has many biological activities (Davis, 2000). Majdi (2014) in studying gene expression of parthenolide biosynthetic pathway in feverfew reported that the expression amount of genes can influence the developmental stage and genotype change. Also, in another report from Majdi (2011), the highest correlation was

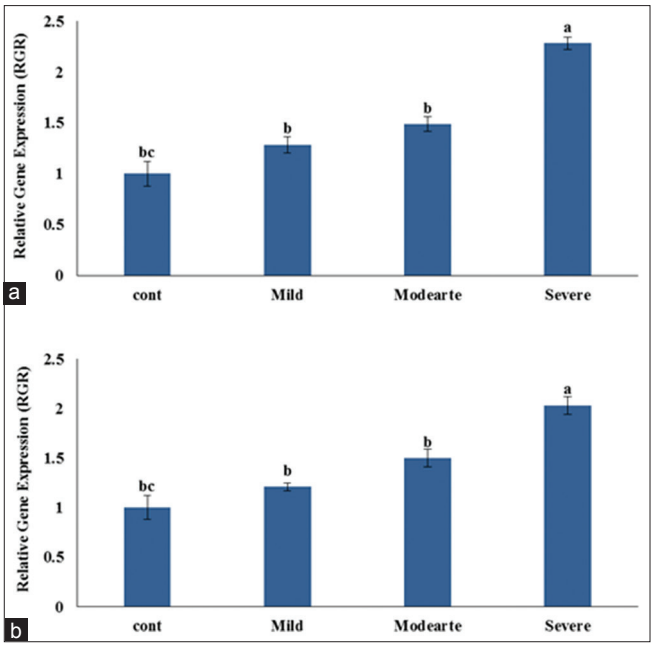

Figure 3: (a) The pattern of TpGAS gene expression in the biosynthesis parthenolide in old leaves of feverfew (the second stage of vegetative phase), (b) The pattern of TpPTS gene expression in the parthenolide biosynthesis pathway in old leaves of feverfew (the second stage of vegetative phase)

observed between the parthenolide amount and Tp GAS gene transcript.

In the present study, it was observed that growth conditions influenced on the expression of genes and Tp GAS and Tp PTS genes expression was reduced in mature leaves in compare to the young leaves (Figure 5) that was consistent with results of Majdi (2011). Different drought treatments (mild, moderate and severe) led to increase in gene expression of PTS and GAS in mature and young leaves compared to control, so that with increasing stress, the gene expression was increased.

Mousavi et al. (2015) in her study on feverfew stated that drought stress as inducer lead to increase gene expression of germacrene A synthase (GAS Tp) and parthenolide synthase (PTS Tp) and parthenolide increase in the plant. Thus, according to the results of this research, it can be said that the expression of GAS and PTS genes affect growth stages and different levels of drought. The drought resulted in increasing the active ingredient of parthenolide in the plant, so that, expression of parthenolide biosynthesis genes are more expressed with increasing drought stress.

CONCLUSION

In the present study, the effects of different levels of drought stress on the expression of key genes affecting

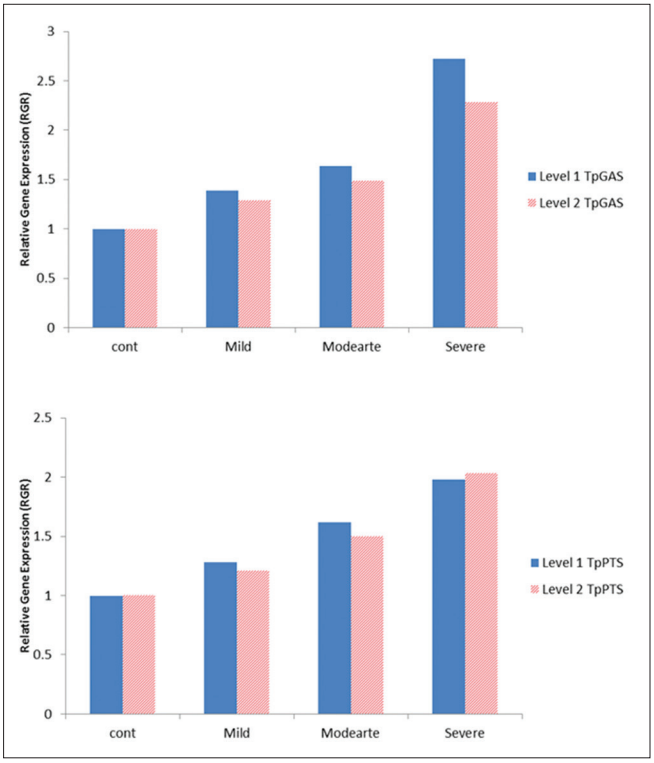

Figure 4: Comparing the pattern of GAS gene expression in parthenolide biosynthesis pathway in young and old leaves of fever few

parthenolide biosynthesis pathway (PTS, GAS) was investigated in the vegetative stage at the young leaves and mature leaves by Real time PCR method. The results showed an increase in expression of studied genes in the treatment under drought stress compared to control both at the growth stage. The highest amount of Tp GAS gene expression was at the first and second stages of growth related to the treatment of severe drought stress (irrigation once every 7 days).

Also, the highest expression of Tp PTS was observed at the first step in the treatment of severe drought and moderate drought stress and in the second vegetative stage (mature leaves) only in severe drought treatment. Comparing the studied gene expression pattern in the first and second stages of vegetative (young and mature leaves) showed a decrease in gene expression in mature leaves rather than the young leaves. Thus, according to this study, it can be considered that drought can be a positive factor in increasing the expression of key genes of the parthenolide biosynthetic pathway and therefore, an increase of parthenolide, so that the largest increase was occurred as a result of maximum drought stress. According to the results, it can be noted that the developmental conditions was effective on genes expression and the young leaves are better source for the active ingredient rather than the adult leaves.

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