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ARTICLE

# Increase of Trigonelline in *Trigonella persica* Plant under Drought Stress

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ABSTRACT

*Trigonella persica* is a valuable medicinal plant which comprises trigonelline that is secondary metabolite and important component in cosmetic and medicine. This research was conducted in order to evaluation the drought stress effect on growth parameters, root anatomical changes and trigonelline content in *T. persica*. Plants were grown under soil moisture corresponding to 100%, 75%, 50% and 25% field capacity for two weeks. The data showed that drought stress was significantly decreased fresh weight and dry weight of shoot and root. In addition, leaf area was declined due to drought stress. Interestingly, root length was enhanced by drought stress. Root microscopic study demonstrated that drought stress increased thickness of epidermal, endodermal, vascular bundle, central cylinder and parenchyma in *T. persica*. Drought stress caused a significant increment in alkaloid and trigonelline content in aerial parts and roots of *T. persica*. These results revealed that *T. persica* responded to drought stress by increasing the alkaloid and trigonelline, as well as the anatomical changes in root. Considering the importance of trigonline and alkaloids, this work may open prospects for production of the pharmaceutically valuable secondary metabolites thereby drought stress.

## 1. Introduction

Drought is one of the most important reasons limiting agricultural production, which extremely influences crop yield [1]. Metabolites play a vital role in plant growth and development. Metabolites are involved in energy storage, cell signaling, membrane construction, and whole plant source distribution under stress conditions. Drought stress change plant metabolism and metabolites thereby metabolic enzyme limitation, substrate scarcity, excess demand for particular combinations, or a combination of these and some other factors [2]. Production of the metabolites by the plants is considered an adaptive ability in coping on stress conditions [3].

There is no doubt that drought stress constantly augments the content of specific plant metabolites. However, growth is considerably declined in drought-stressed plants as well; thus, the decrease in biomass could result in the enhanced content of specialized plant metabolites [4,5]. In some recent reports, it had been shown that drought stress promoted production of secondary metabolites include

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phenols and terpenes in plants [6,7]. Alkaloids are the most diverse class among nitrogenous combinations. Alkaloids have numerous biological activities, and there are some drugs accessible on the market manufactured from natural plant alkaloids [8]. The content of total alkaloid in stems of *Dendrobium moniliforme* enhanced under drought stress [9]. Yahyazadeh et al. [10] showed that drought stress increased alkaloid content in *Chelidonium majus* L.

Plant roots, as organs that directly uptake water, play an important role in drought stress. Developed roots can aid plants to completely uptake and consume the stored water in the soil so that plants can live the drought period. Configuration of root system include root branches, root hair and root density can significantly influence the water shortage of plants [11]. Anatomical alterations may occur in roots under drought to maintain and adapt the plants to this stress. Root anatomical properties influence axial and radial water transport in roots, which would influence the efficiency of water absorption and distribution. Cortical characters and the existence of suberized cell layers may influence radial conductance while Xylem vessel characters (diameter, number,and area) influence axial water conductance [12]. Moderate drought enhanced aerenchyma development in root cortex but decreased the diameter and number of xylem vessels in the vascular cylinder in *Typha domingensis* [13].

The genus Trigonella is one of the largest genera of the tribe Trifoliatae in the family Fabaceae and sub-family Papilionaceae [14]. Leaves, stems and seeds of trigonella are consumed in various countries around the world for various goals such as decreasing blood sugar, lowering cholesterol level, anti-diabetic, anti-microbial, anti-cancer, etc. [15]. The pharmacological and biological properties of the trigonella are attributed to the variety of its components such as N-compounds, steroids, amino acids, polyphenolic constituents, and volatile constituents [16]. *Trigonella persica* Boiss is the only endemic species in Iran [17]. Trigoneline is an important metabolite in *T. persica*. Trigoneline or N-methyl nicotinic acid is a secondary metabolite derived from pyridine nucleotides [18]. Trigonelline is considered as a physiologically active constituent in plants which can cause the leaf movements and act as an osmoregulator and osmoprotectant in response to abiotic stresses [19].

Investigating the anatomical and architectural properties that contribute to rooting depth is necessary for prompting crop performance under drought stress. Thus, the aim of this work was to study the impacts of various levels of drought on the root anatomy of *T. persica*. The information will be useful for evaluation of root anatomy associated to drought tolerance and choice of important traits for drought tolerance. There are no data on how drought conditions influence on trigonelline accumulation in *T. persica*. Thus, the other aim of this work was to study how drought stress impacts the content of trigonelline in *T. persica*.

## 2. Materials and Methods

### Plant growth condition and drought treatment

Seeds of *T. persica* were obtained from Semirom Agricultural Institute, Isfahan, Iran, and used for the experiments. The seeds of *T. persica* were sterilized by hypochlorite for 15 min and then rinsed with distilled water. The sterilized seeds were sown in plastic pots. The pots contained autoclaved soil. Soil properties were determined using XRF (PW 2404, Philips, and Netherland) the results of which are shown in Table 1.

**Table 1.** Physico-chemical properties of the experimental soil

| Soil texture | pH | Zn (%) | Rb (%) | Ba (%) | Zr (%) | Sr (%) |
|---|---|---|---|---|---|---|
| Loam-clay | 7.8 | 0.079 | 0.008 | 0.066 | 0.017 | 0.041 |

The pots were put in the Phytotron system (at temperature 25 °C and humidity 40%). Also, in this system, 16 h of light (combined of fluorescence and filament lamp) and 8 h of darkness were provided during the growth period (at a photon fluence rate of 3.35 μmol $m^{-2}$ $s^{-1}$). After forty days, the pots were divided into four groups. There were three replicates per treatment and the plastic pots were arranged into completely randomized block design in a factorial arrangement. Forty day-old plants were irrigated to 100%, 75%, 50%, and 25% FC for two weeks. Irrigation was performed three times per week. Two weeks after water deficit, plants were collected for analyses in all the experiments.

### Determination of growth traits

To determinate fresh weight (FW), root, leaf and stem samples were washed off with water to remove soil and blotted gently with soft paper towel to remove any free surface moisture. Fresh weights were determined immediately and dry weights (DW) were measured after drying in an oven at 60 °C for 48 h. Saturated masses of fresh tissues were determined by keeping them in water for 24 h, followed by drying in an oven at 60 °C for 48 h until constant weight was achieved.

To measuring the relative water content (RWC) of leaves, the FW of the leaves was first measured, then the leaves were immersed in distilled water for 48 h in the dark at 4 °C and their saturation weight (SW) was measured. The leaves were then placed in an oven at 60 °C for

48 h and their DW was measured [20]; RWC is calculated from the following equation:

$$RWC\ (\%) = [(FW-DW)/(SW-DW)]100$$

### Determination of morphoanatomical parameters in roots

Root samples were fixed in formalin 37%-acetic acid 100%-ethanol 95% (FAA and stored at 4 °C until sectioning. Transverse sections were obtained using a rotating microtome. The sections were stained with methyl blue and methyl green 1% for cellulose and lignin, respectively. Samples were mounted in glycerol and examined with an Olympus microscope (BH 2). Morphoanatomical parameters including, the diameter of root, root epidermis, root cortex and root central cylinder were measured. The measurements were carried out using Image J Software.

### Total alkaloid assessment

The content of total alkaloid was determined depending on the method which was described as follows. 1 g dry weight was soaked with 80 mL acetic acid (0.05%), and they were sealed for 18 h. The resulting solution was passed through Whatman No. 3 filter paper. They were washed with chloroform for several times. In the next step, the soluble pH was reached about 7 using ammonia. 10 mL sample solution was put in the 50-mL centrifuge tube and 5 mL potassium buffer was added. Then 5 mL bromocresol green solution. The resulting solution was extracted with 5 mL, 8 mL, and 10 mL of chloroform. The chloroform phase solution was transferred to a 25 mL balloon and then reached volume by chloroform. Finally, absorbance of solution was read at 415 nm. The total alkaloid content was calculated by using atropine standard.

### Trigoneline measurement

Dry tissues (0.5 g) of plants were ground with methanol in a mortar and pestle. After incubation at 25 °C for 22 h, the homogenates were centrifuged at 3000 rpm and the supernatant was collected. After complete evaporation of the methanol by rotary evaporator, the methanol-soluble extracts were dissolved in 1 mL methanol. The samples after filtering were used for determination of trigonelline by HPLC [21]. The HPLC system equipped with a reverse-phase C18 column (OSD-UG-5, 4.6 mm × 250 mm × 10 µm) and UV detector. Samples were eluted using the mobile phase of methanol: distilled water (50:50, v/v), adjusted to pH 5 with hydrochloric acid and delivered at a flow rate of 1.0 mL/min. Detection was carried out at 267 nm at room temperature (25 ± 1 °C). The injection volume was 40 µL for all runs. The trigonelline accumulation was determined using a calibration curve compared with standards and a co-chromatogram of the standards and samples. Standard cure and chromatogram of trigonelline were showed in Figure 1.

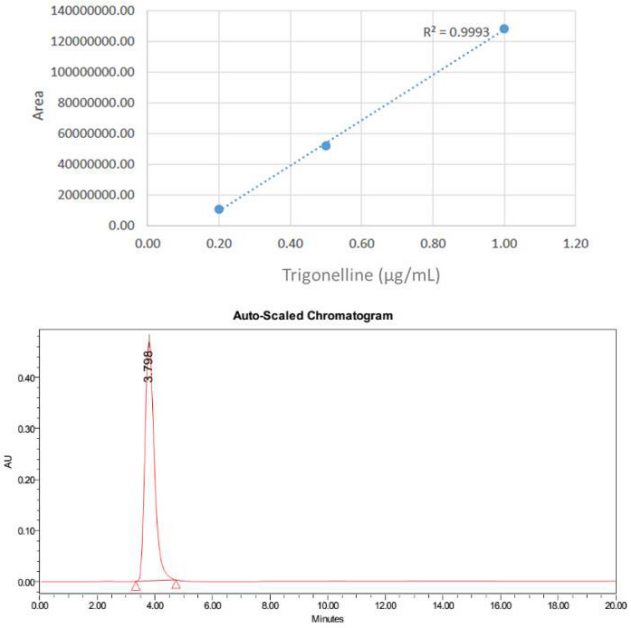


**Figure 1.** Standard cure and chromatogram of trigonelline.

### Statistical methods

All data were analyzed with statistical software SPSS version 19. The means were compared by Duncan's test at the 0.05 level of confidence.

## 3. Results and Discussion

Drought stress is one of the main abiotic stresses that influences plant growth and biomass production. Roots and shoots were followed by measuring length, FW and DW in *T. persica* plants under different drought treatments. The results shown in Table 2 indicated that drought stress deteriorated all growth parameters include shoot length, shoot FW, shoot DW, root FW, root DW, leaf area and RWC, except root length as compared to control. The largest decrease in growth parameters occurred at 25% FC. These consequences might be owing to the declination of photosynthesis, reduced cell turgidity, enhanced evapotranspiration, declined $CO_2$ assimilation due to stomatal closure, and finally, decreased cell division under drought stress [22,23]. Enhancement of root length was as a defense strategy to deal with drought conditions. A similar decrease in growth has been previously witnessed under drought stress in *Fagopyrum tataricum* [24]. Rezayian et al. [25] showed that drought stress reduced growth in *Brassica napus*.

**Table 2.** Effect of different irrigation levels on growth parameters in *T. persica* plant

| Field capacity (%) | Shoot length (cm) | Shoot FW (mg) | Shoot DW (mg) | Root length (cm) | Root FW (mg) | Root DW (mg) | Leaf area ($cm^2$) | RWC (%) |
|---|---|---|---|---|---|---|---|---|
| **100** | 20.26±1.50 a | 82±6.24 a | 4.03±2.95 a | 4.8±0.70 b | 21±2.22 ab | 8±1.30 ab | 4.32±0.99 a | 67.89±2.11 a |
| **75** | 13.06±2.01 ab | 52±5 b | 1.60±1.21 a | 5.06±0.70 b | 25±1.11 a | 9±0.99 a | 1.49±0.12 b | 49.57± 1.2 ab |
| **50** | 11.66±1.2 b | 64±6 b | 6.33±5.50 a | 5.56±0.81 ab | 18±1.01 c | 6±0.87 c | 1.61±0.32 b | 38.67±2.45 bc |
| **25** | 11.93±1.65 b | 60.33±7.7 b | 1.60±1.31 a | 6.83±0.76 a | 10± 1.06 c | 5±0.43 c | 0.43±0.08 c | 21.47±1.09 c |

Mean ± SE based on three replicates for growth parameters are presented

Root traits influence the amount of nutrient and water absorption, and are essential for preserving crop yield under drought conditions [26]. Cross section of *T. persica* root was studied to assess the anatomical adaptations of this plant to be acclimatized under drought stress. There were significant changes in anatomical features of root of *T. persica* plants imposed to various levels of drought (Figure 2 and Table 3). By examining the cross sections of root from control and treated plants, it was observed that thickness of epidermal and endodermal layer was increased as compared to control. On the other hand, thickness of central cylinder and parenchyma enhanced in drought-treated plants. The vascular bundle of drought-exposed plants was significantly greater in diameter and number than the controls. According to obtained data, drought caused a significant increment in the root diameter in plants exposed to drought stress compared with the controls.

**Table 3.** The results of anatomical measurements of *T. persica* root under different irrigation levels

| Field capacity (%) | Epidermal thickness (µm) | Endodermal thickness (µm) | Central cylinder thickness (µm) | Parenchyma thickness (µm) | Vascular bundle number | Vascular bundle diameter (µm) | Root diameter (µm) |
|---|---|---|---|---|---|---|---|
| **100** | 9.53±1.50 b | 1.81±0.24 c | 138.23±2.15 b | 303.33±6.70 c | 3.33±0.57 c | 138.33±18.9 b | 783±8 c |
| **75** | 15.53±2.01 a | 3±0.90 b | 182±3.21 a | 338.66±2.70 b | 4.33±1.52 c | 182±12.12 a | 829±19 b |
| **50** | 15±1.52 a | 2.5±0.96 b | 189±2.50 a | 383.66±2.61 a | 6.66±1.15 b | 189±14.93 a | 66.3 ±15 a |
| **25** | 14.66±2.65 a | 4.23±0.87 a | 146±3.31 b | 351±3.56 a | 8.66±1.15 a | 146±23.71 b | 824±18 b |

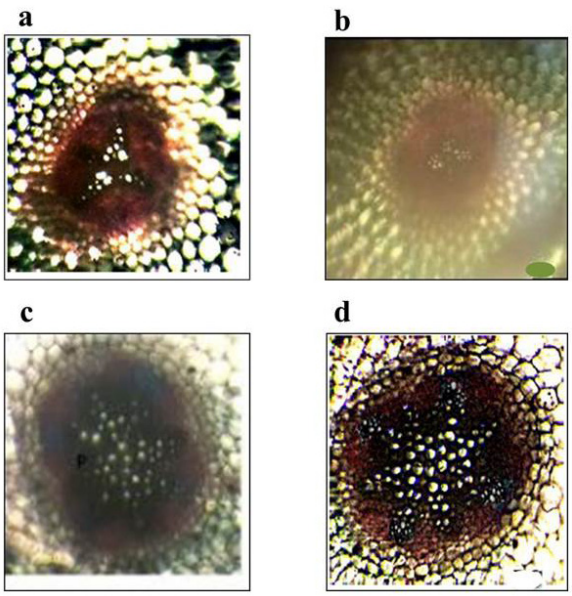


**Figure 2.** Cross sections of the roots in the drought-treated *T. persica* plants.
a (100% FC), b (75% FC), c (25% FC), d (50% FC).

Under drought conditions, roots expand to aid extract soil moisture which being held at larger surface tension [27]. Also, deep root growth and xylem diameter in roots may enhance the capacity of roots to mine more water in deep soil when water in deep soil is abundant. Roots with greater length support plants to enhance water absorption and conserve plant biomass under drought conditions by enhancing surface area and root length in contact with soil water [28,29].

Our findings propose a complex network of anatomical adaptations such as with increased epidermal and endodermal thickness, enhanced vascular bundle, augmented root diameter end etc. These proprieties are required for the conservation of water potential and energy storage under drought stress which can develop the resistance of *T. persica* to survive in drought conditions. The improvement of endodermal layers around the stele are considered mean to avoid the dryness of meristematic tissues [30]. In this study, root diameter enhanced in *T. persica under* drought condition, it has direct relation with water uptake and has more capacity to explore soil [31,32]. Numerous studies have reported the significance of root system for absorption of water from soil layers under drought condition in different plants such as rice (*Oryza sativa* L.) [33] and wheat [34].

In this experiment, an increase in the production of total alkaloid was observed in aerial parts and roots when *T. persica* plants were kept under severe drought conditions (Figure 3a,b). Our data suggest that the cultivation of *T. persica* in severe drought stress would serve as suitable treatment for accumulating alkaloid. The fact that drought stress causes increases in particular metabolism constituents such as alkaloids has been shown by some authors, such as Ghorbanpour et al. [35] in work with *Hyoscyamus niger*; and Kleinwächter et al. [36] with spices. The up-regulation of the biosynthesis of alkaloids may contribute to decline in the reducing status of the electron transport chain under stress conditions. These compounds have a further role in the dissipation of excess energy and thus inhibit the generation of toxic oxygen radicals [10,37]. Liu et al. [38] stated that drought stress enhanced alkaloid content by increasing the expression of genes involved in their biosynthesis.

Trigonelline content was quantified in aerial parts and roots of *T. persica* plants under drought conditions (Figure 4a,b). The findings demonstrated that trigonelline content increased in the plants exposed to moderate (5% FC) and severe (25% FC) drought stress. An increase in trigonel-

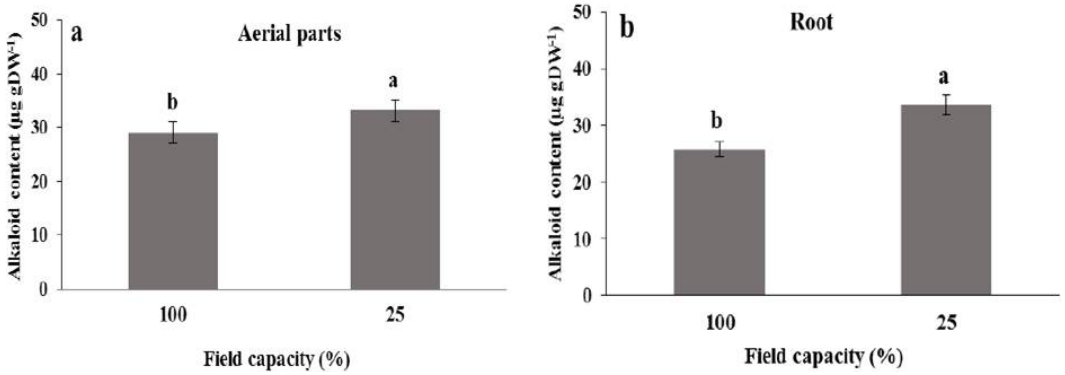


**Figure 3.** Effect of drought stress on alkaloid content in aerial parts (a) and root (b) of *T. persica*. Values are means ± SE of three replicates. Different letters indicated significant ($p<0.05$) differences.

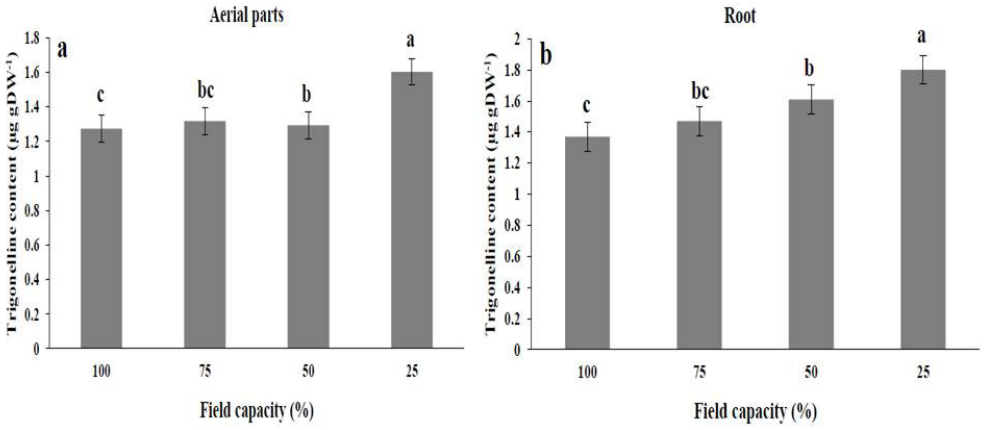


**Figure 4.** Change in trigonelline content in aerial parts (a) and root (b) of *T. persica* by different drought stress. Values are means ± SE of three replicates. Different letters indicated significant ($p<0.05$) differences.

line content in drought conditions compared to control supports the protecting role of this biomolecule under unsuitable environmental conditions. This increase in severe drought level was higher compared to moderate drought level. Possibly, the more accumulation of secondary metabolites in plants in a stressed environment occurred to inhibit too enormous production of reactive oxygen species (ROS) and corresponding injuries by photoinhibition [4,39]. Some studies have presented the role of trigonelline in the mechanism of plant defense [40,41]. Dadrasan et al. [42] and Zamani et al. [43] reported that drought stress enhanced trigonelline accumulation in *T. foenum-graecum*. The demand for secondary metabolites from plants for the medicinal industry coupled with the low yields necessitate abiotic or biotic factors augmenting the potential to produce beneficial phytochemicals. Therefore, due to the importance of trigonelline, drought stress can be used for the accumulation of this valuable substance in *T. persica plant.*

In conclusion, this study showed that drought stress decreased *T. persica* growth and induced changes in anatomical characteristics such as thickness of epidermal, endodermal, vascular bundle, central cylinder and parenchyma in *T. persica* roots. Drought stress enhanced alkaloid content and trigonelline content in *T. persica* plants. Therefore, it is possible to use this stress to increase the valuable metabolites in this plant.

## Conflicts of Interest

The authors declare no potential conflicts of interest regarding the publication of this paper.

## References

[1] Khan, M.A., Iqbal, M., Akram, M., et al., 2013. Recent advances in molecular tool development for drought tolerance breeding in cereal crops: a review. Zemdirbyste-Agriculture. 100(3), 325-334.

[2] Wen, W., Li, K., Alseek, S., et al., 2015. Genetic determinants of the network of primary metabolism and their relationships to plant performance in a maize recombinant inbred line population. Plant Cell. 27(7), 1839-1856.

[3] Edreva, A., Velikova, V., Tsonev, T., et al., 2008. Stress-protective role of secondary metabolites: diversity of functions and mechanisms. General & Applied Plant Physiology. 34(1-2), 67-78.

[4] Selmar, D., Kleinwächter, M., 2013. Influencing the product quality by deliberately applying drought stress during the cultivation of medicinal plants. Industrial Crops and Products. 42, 558-566.

[5] Paulsen, J., Selmar, D., 2016. Case study: the difficulty of correct reference values when evaluating the effects of drought stress: a case study with Thymus vulgaris. Journal of Applied Botany & Food Quality. 89, 287-289.

[6] de Matos Nunes, J., Bertodo, L.O.O., Da Rosa, L.M.G., et al., 2014. Stress induction of valuable secondary metabolites in Hypericum polyanthemum acclimatized plants. South African Journal of Botany. 94, 182-189.

[7] Niinemets, Ü., 2016. Uncovering the hidden facets of drought stress: secondary metabolites make the difference. Tree Physiology. 36(2), 129-132.

[8] Debnath, B., Singha, W.S., Dasa, M., et al., 2018. Role of plant alkaloids on human health: A review of biological activities. Materials Today Chemistry. 9, 56-72.

[9] Wu, X., Yuan, J., Luo, A., et al., 2016. Drought stress and re-watering increase secondary metabolites and enzyme activity in *dendrobium moniliforme*. Industrial Crops & Products. 94, 385-393.

[10] Yahyazadeh, M., Meinen, R., Hänsch, R., et al., 2018. Impact of drought and salt stress on the biosynthesis of alkaloids in *Chelidonium majus* L. Phytochemistry. 152, 204-212.

[11] Gowda, V.R.P., Henry, A., Yamauchi, A., et al., 2011. Root biology and genetic improvement for drought avoidance in rice. Field Crops Research. 122(1), 1-13.

[12] Lynch, J.P., 2015. Root phenes that reduce the metabolic costs of soil exploration: opportunities for 21st century agriculture. Plant, Cell & Environment. 38(9), 1775-1784.

[13] da Cunha Cruz, Y., Scarpa, A.L.M., Pereira, M.P., et al., 2020. Root anatomy and nutrient uptake of the cattail Typha domingensis Pers.(Typhaceae) grown under drought condition. Rhizosphere. 16, 100253.

[14] Balodi, B., 1991. The genus Trigonella L.(Fabaceae) in the northwest Himalaya. Journal of Economic and Taxonomic Botany. 5, 11-16.

[15] Al-Habori, M., Raman, A., 2002. Pharmalogical properties in Fenugreek: The genus Trigonella. Petropoulos, GA. Taylor and Francis. London.

[16] Bano, D., Tabassum, H., Ahmad, A., et al., 2016. The medicinal significance of the bioactive compounds of Trigonella foenum-graecum: a review. International Journal of Research in Ayurveda and Pharmacy. 7, 84-91.

[17] Rechinger, K.H., 1984. Rumex (Polygonaceae) in Australia: a reconsideration. Nuytsia.

[18] Mehrafarin, A., Ghaderi, A., Rezazadeh, S.H., et al., 2010. Bioengineering of important secondary metabolites and metabolic pathways in fenugreek (Trigonella foenum-graecum L.).

[19] Evans, L.S., Tramontano, W.A., 1984. Trigonelline and promotion of cell arrest in G2 of various le-

gumes. Phytochemistry. 23(9), 1837-1840.

[20] Altinkut, A., Kazan, K., Ipekci, Z., et al., 2001. Tolerance to paraquat is correlated with the traits associated with water stress tolerance in segregating F2 populations of barley and wheat. Euphytica. 121(1), 81-86.

[21] Shailajan, S., Sayed, N., Menon, S., et al., 2011. A validated RP-HPLC method for quantitation of trigonelline from herbal formulations containing Trigonella foenum-graecum (L.) seeds. Pharmaceutical Methods. 2(3), 157-160.

[22] Rahimi, Y., Taleei, A., Ranjbar, M., 2017. Changes in the expression of key genes involved in the biosynthesis of menthol and menthofuran in Mentha piperita L. under drought stress. Acta Physiologiae Plantarum. 39(9), 1-9.

[23] Karimi, S., Yadollahi, A., Arzani, K., et al., 2015. Gas-exchange response of almond genotypes to water stress. Photosynthetica. 53(1), 29-34.

[24] Hossain, M.S., Li, J., Sikdar, A., et al., 2020. Exogenous melatonin modulates the physiological and biochemical mechanisms of drought tolerance in tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn). Molecules. 25(12), 2828.

[25] Rezayian, M., Niknam, V., Ebrahimzadeh, H., 2018. Positive effects of Penconazole on growth of *Brassica napus* under drought stress. Archives of Agronomy and Soil Science. 64(13), 1791-1806. DOI: https://doi.org/10.1080/03650340.2018.1458095

[26] Narayanan, S., Mohan, A., Gill, K.S., et al., 2014. Variability of root traits in spring wheat germplasm. PLoS One. 9(6), e100317.

[27] Comas, L.H., Mueller, K.E., Taylor, L.L., et al., 2012. Evolutionary patterns and biogeochemical significance of angiosperm root traits. International Journal of Plant Sciences. 173(6), 584-595.

[28] Comas, L., Becker, S., Cruz, V.M.V., et al., 2013. Root traits contributing to plant productivity under drought. Frontiers in Plant Science. 4, 442.

[29] Thangthong, N., Jogloy, S., Pensuk, V., et al., 2016. Distribution patterns of peanut roots under different durations of early season drought stress. Field Crops Research. 198, 40-49.

[30] North, G.B., Nobel, P.S., 1992. Drought-induced changes in hydraulic conductivity and structure in roots of Ferocactus acanthodes and Opuntia ficus-indica. New Phytologist. 120(1), 9-19.

[31] Bao, Y., Aggarwal, P., Robbins, N.E., et al., 2014. Plant roots use a patterning mechanism to position lateral root branches toward available water," Proceedings of the National Academy of Sciences. 111(25), 9319-9324.

[32] Richards, R.A., Condon, A.G., Rebetzke, G.J., 2001. Traits to improve yield in dry environments. Application of Physiology in Wheat Breeding.

[33] Wasson, A.P., Richards, R.A., Chatrath, R., et al., 2012. Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. Journal of Experimental Botany. 63(9), 3485-3498.

[34] Kashiwagi, J., Krishnamurthy, L., Crouch, J.H., et al., 2006. Variability of root length density and its contributions to seed yield in chickpea (Cicer arietinum L.) under terminal drought stress. Field Crops Research. 95(2-3), 171-181.

[35] Ghorbanpour, M., Hatami, M., Khavazi, K., 2013. Role of plant growth promoting rhizobacteria on antioxidant enzyme activities and tropane alkaloid production of *Hyoscyamus niger* under water deficit stress. Turkish Journal of Biology. 37(3), 350-360.

[36] Kleinwächter, M., Paulsen, J., Bloem, E., et al., 2015. Moderate drought and signal transducer induced biosynthesis of relevant secondary metabolites in thyme (Thymus vulgaris), greater celandine (*Chelidonium majus*) and parsley (Petroselinum crispum). Industrial Crops & Products. 64, 158-166.

[37] Falk, K.L., Tokuhisa, J.G., Gershenzon, J., 2007. The effect of sulfur nutrition on plant glucosinolate content: physiology and molecular mechanisms. Plant Biology. 9(5), 573-581.

[38] Liu, Y., Meng, Q., Duan, X., et al., 2017. Effects of PEG-induced drought stress on regulation of indole alkaloid biosynthesis in Catharanthus roseus. Journal of Plant Interactions. 12(1), 87-91.

[39] Keshavarz Afshar, R., Chaichi, M.R., Ansari Jovini, M., et al., 2015. Accumulation of silymarin in milk thistle seeds under drought stress. Planta. 242(3), 539-543.

[40] Cho, Y., Lightfoot, D.A., Wood, A.J., 1999. Trigonelline concentrations in salt stressed leaves of cultivated Glycine max. Phytochemistry. 52(7), 1235-1238.

[41] Rajasekaran, L.R., Aspinall, D., Jones, G.P., et al., 2001. Stress metabolism. IX. Effect of salt stress on trigonelline accumulation in tomato. Canadian Journal of Plant Science. 81(3), 487-498.

[42] Dadrasan, M., Chaichi, M.R., Pourbabaee, A.A., et al., 2015. Deficit irrigation and biological fertilizer influence on yield and trigonelline production of fenugreek. Industrial Crops & Products. 77, 156-162.

[43] Zamani, Z., Amiri, H., Ismaili, A., 2020. Improving drought stress tolerance in fenugreek (Trigonella foenum-graecum) by exogenous melatonin. Plant Biosystems An International Journal Dealing with All Aspects of Plant Biology. 154(5), 643-655.