Journal of Agricultural Science and Technology (JAST), 29(2)
In Press, Pre-Proof Version

Impacts of Drought Stress on Morphine Biosynthesis in *Papaver somniferum*

Ahad Yamchi[1], Ali Asghar Nasrollahnezhad Ghomi[1], Shokooh Hemati[1], and Seyedeh Sanaz Ramezanpour[1]

Abstract

Papaver somniferum L. represents an esteemed medicinal flora that encompasses benzylisoquinoline alkaloids (BIAs), such as codeine, morphine, berberine, sanguinarine, and papaverine. The expression levels of three pivotal genes (T6ODM, COR, and CODM) implicated in the morphine biosynthetic pathway were scrutinized in capsule cells subjected to varying degrees of drought stress. Poppy seeds were cultivated in pots within a greenhouse environment, and drought treatments were instituted during the flowering phase. Capsules were collected at distinct drought stress gradients, encompassing field capacity (FC) of 100% (control), FC 80%, FC 60%, FC 40%, and FC 20% (stress treatment). This investigation was executed employing a completely randomized design with three replications. The experimental treatments encompassed differing intensities of drought stress, and the parameters evaluated included gene expression and alterations in morphine concentration within poppy capsule cells. RNA was isolated from the specimens, and complementary DNA (cDNA) was synthesized. The expression of the *T6ODM* gene exhibited a significant elevation after stress at 40% FC compared with control specimens ($p< 0.01$). In contrast, the expression levels of the *COR* and *CODM* genes declined across all drought stress conditions when juxtaposed with control specimens ($p< 0.01$). The variation in morphine concentration in capsule cells under disparate drought stress conditions paralleled the expression of the *T6ODM* gene, demonstrating an uptick in morphine following stress at 40% FC ($p< 0.01$). Furthermore, Pearson correlation analysis disclosed a substantial correlation between morphine content and the expression of the *T6ODM* gene ($p< 0.01$) in the capsule cells.

Keywords: *CODM* gene, *COR* gene, Poppy plant, *T6ODM* gene, Water stress.

Introduction

Plant alkaloids are crucial natural organic substances that play vital roles in plant growth, development, and defense strategies (Pereira et al., 2023). Their unique biological properties have

[1] Department of Plant Breeding and Biotechnology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Islamic Republic of Iran.
*Corresponding author; e-mail: nasrollahnejad@gau.ac.ir

garnered significant interest in pharmaceutical development and agricultural practices (Azizyan et al., 2024; Li et al., 2023).

Opium, found in the latex-dried poppy capsule, contains over 80 isoquinoline alkaloids. The most common alkaloid derived from opium is morphine (4-21%), followed by codeine, thebaine, papaverine, noscapine, and narcine (Dewick, 2002). Alkaloids are a group of nitrogenous compounds with a low molecular weight that are found in 20% of plant species. Some alkaloids have been used as drugs, stimulants, and toxins due to their strong biological activity (Facchini, 2001). Benzyl isoquinoline alkaloids are another large group of natural products with about 2,500 compounds found primarily in the Papaveraceae, Ranunculaceae, Berberidaceae, and Menispermaceae families of plants (Jacobs, 2004). Alkaloids are formed and accumulated in specific cells and organs of the plant. Morphine, noscapine, and papaverine are common alkaloids found in aerial organs, whereas sanguinarine is typically found in roots (Facchini and DeLuca, 1995). Alkaloids accumulate first in the roots, then in the seedling stage, and finally in all aerial organs.

The reference genome of opium poppy, with a size of 2.72 Gb carrying 51,213 protein-coding genes and 9,494 non-coding RNAs, was reported (Guo et al., 2018). The investigation of protein–protein interactions and pivotal hub genes reveals a multitude of components engaged in metabolic and redox regulatory functions, with functional analyses of critical hub genes—such as those from the thioredoxin family, Fe2OG dioxygenase domain proteins, iron/ascorbate-dependent oxidoreductase family SCP domain proteins, and salutaridinol 7-O-acetyltransferase—emphasizing their diverse roles in redox regulation and various biological activities, thus highlighting their essential contributions to morphine biosynthesis dynamics (Bagheri et al., 2025). After the petals have fallen, measurements of alkaloids in capsules collected from the poppy plant indicate that morphine alkaloids are present (Shukla and Singh, 2000). Morphine and codeine are more concentrated in aerial organs, particularly in the poppy capsule (Facchini et al., 2007). The conversion of thebaine to neopinone occurs via the enzymatic action of 6-O-demethylase (T6ODM) within the morphine biosynthetic pathway. Following this transformation, neopinone undergoes a spontaneous reorganization, culminating in the formation of codeine (Hagel and Facchini, 2010). The enzyme codeinone reductase (COR) facilitates the reduction of codeinone to codeine (Unterlinner and Kutchan, 1999). Subsequently, codeine is transformed into morphine

Journal of Agricultural Science and Technology (JAST), 29(2)
In Press, Pre-Proof Version

through the action of codeine-O-demethylase (CODM). Alternatively, CODM also mediates the conversion of thebaine to oripavin, which constitutes the initial step in the associated sub-pathway. The compound oripavin is then converted to morphinane by T6ODM and is ultimately reduced to morphine by COR (Figure S1). In instances where the poppy plant experiences mechanical damage, morphine is rapidly metabolized into bis-morphine. Its binding to the cell wall enhances resistance to pectinase hydrolysis, suggesting that morphine may serve a defensive function against biotic stresses (Morimoto et al., 2001). Davoudnia et al. (2016) investigated alterations in gene expression, notably those of the Berberine bridge enzyme (BBE1), Scoulerine-O-demethylase (DIOX2), and Dihydrobenzophenanthridine oxidase (DBOX), that are implicated in the sanguinarine biosynthetic pathway within the Papaver genus under conditions of drought and salinity stress. Their findings indicated that the upregulation of BBE1, DIOX2, and DBOX genes in the poppy plant was significantly more pronounced under drought conditions compared with salinity (Davoudnia et al., 2016).

In response to drought conditions, plants engage in intricate physiological and molecular adaptive processes (Sun and Fernie, 2024). Such adaptations encompass mechanisms for water conservation, osmotic regulation, and the activation of antioxidant systems, in addition to the biosynthesis of secondary metabolites (Cao et al., 2024; Palesh et al., 2020). Alkaloids serve as vital defensive compounds for plants, contributing to both survival and reproductive success by deterring herbivory and facilitating pollinator attraction (Zhang et al., 2024). These compounds represent significant bioactive constituents within various esteemed medicinal plants, exhibiting extraordinary pharmacological attributes, which include notable examples such as leonurin, dendrobine, ephedrine, triptolide, and scopolamine. The pharmacological effects of these alkaloids encompass neuroprotective, antitumor, anti-inflammatory, antibacterial, and antiviral activities (Bhardwaj et al., 2024; Duda-Madej et al., 2024; Li et al., 2024a). Nonetheless, drought stress negatively impacts the growth and quality of medicinal plants, potentially leading to reductions in alkaloid concentrations and hindering advancements within the industry (Feng et al., 2024). A comprehensive understanding of the regulatory mechanisms underlying plant alkaloid biosynthesis in response to stressors will enhance insights into plant adaptive responses to drought and significantly influence the development of pharmaceuticals utilizing alkaloidal resources (Guedes et al., 2024).

Journal of Agricultural Science and Technology (JAST), 29(2)
In Press, Pre-Proof Version

There exists a limited body of research addressing the effects of drought stress on the molecular metabolism of *Papaver somniferum* (Özcan et al., 2025), indicating a pressing need for further investigation aimed at augmenting the secondary metabolites of plants within the *Papaver* genus, which harbor compounds of medicinal significance and are integral to the treatment of numerous diseases.

The objective of the present study was to evaluate morphine accumulation and to ascertain the expression levels of three pivotal genes (*T6ODM, CODM*, and *COR*) that are involved in the morphine biosynthetic pathway within capsule cells subjected to drought stress, as well as to assess the correlation between morphine concentration and the expression of these related genes.

Materials and Methods

Plant material, culture conditions, and statistical analysis

The National Center for Genetic and Biological Resources of Iran provided *Papaver somniferum* species with identification number P1006760 to begin poppy seed research. The seeds were stored in a damp cloth in the refrigerator for 24 hours before planting to break the seed dormancy. This experiment was conducted in a Completely Randomized Design with three replications. Under natural light and at a temperature of 20-25 ° C, seeds were planted in pots with equal volumes of leaf soil, garden soil, and aerated sand in the greenhouse of Gorgan University of Agricultural Sciences and Natural Resources (Figure S2).

Drought stress treatments

Drought stress was applied to plants during the flowering stage, three days after the petals fell. The capsule was sampled when the moisture in the pots was at different levels of drought stress, including field capacity (FC) at 100% (as a control), 80%, 60%, 40%, and 20% (as stress treatments). The sampling at four FC levels (20-80%) was compared to the control plant (FC 100%). Finally, the samples were wrapped in aluminum foil and frozen in liquid nitrogen before being stored at - 80 °C.

Primer design

Primers were designed by Allele ID 7.5 (Table 1). When possible, they were designed to include the 3′ untranslated region or the last exon. Primer specificity was checked by looking for homologies in the *P. somniferum* genome using the BLASTn tool.

Journal of Agricultural Science and Technology (JAST), 29(2)
In Press, Pre-Proof Version

Table 1. Information and sequence of primers related to *T6ODM*, *CODM*, and *COR* genes.

Initiator name	Sequence	Tm (°C)	Product length	Accession number of the genes
COR For	5′ AAAACTCCCAGTGCCTCTCA 3′	63.5	115bp	FJ624147
COR Rev	5′ ACCCTTAATCTCGGCTGCTT 3′	63.5		
CODM For	5′ TTGTGCTTAAATTTCGTGGATGAC 3′	60.1	129bp	GQ500141
CODM Rev	5′ TGATTACATCACTTGACCCAAACAG 3′	62.5		
T6ODM For	5′ TTGATTGGGAACTAACGGCAGAAG 3′	58.4	124bp	GQ500139
T6ODM Rev	5′ TGAAAGGTCCAGTCGGTGATAACA 3′	58.4		
β-ACTIN For	5′ TCTCAACCCAAAGGCTAATCG 3’	59.4	143bp	RZC79158
β-ACTIN Rev	5′ CCCCAGAATCCAAGACAATAC 3’	59.4		

RNA extraction, RT-PCR, and qPCR reaction

A 200 mg tissue sample was used for total RNA extraction by p-BIOZOL buffer (BioFlux- Japan). The extracted RNA was run on a TBE agarose gel (1% W/V) by electrophoresis to determine the quality of the RNA. Five microliters of extracted RNA (500 ng) were treated with DNase I, and the first cDNA strand was synthesized with the reverse transcription system using an Oligo-dT primer according to Thermo Scientific company instructions. The qPCR reaction was performed in a volume of 10 µL containing cDNA (5 ng), 0.6 µM of each primer, and 5 µL of SYBR Green master mix (2X) (Applied Biosystems). Real-time reactions were carried out on an iQ5 (Bio-Rad, USA) machine. The program of qPCR was 94 °C for 3 min, followed by 40 cycles of 94°C for 10s, 62°C for 30s, and 72°C for 30s. Reactions were finished with a dissociation step of 95 °C for 15 s, T_a (°C) for 15 s, and 95 °C for 15 s. Real-time PCR experiments were done in three biological repeats, each with three technical replications. The beta-actin host gene was used as an internal control gene. The Lin Reg v.4 software was used to select primers with efficiency values that exhibit $R^2 > 0.98$. Relative gene expression levels were calculated by the comparative method (the $2^{-\Delta\Delta Ct}$ method) (Schmittgen et al., 2000) using GenEX software, and the graph showing the expression of each gene in the capsule tissue was generated in Excel. The specificity of the RT-qPCR product was evaluated by the dissociation curve of the reaction as well as electrophoresis of the product on TBE agarose gel (1% W/V).

Alkaloid extraction

Alkaloid extraction was performed using the Facchini and DeLuca methods with minor modifications (Facchini and DeLuca, 1995; Frick et al., 2004). One gram of powdered poppy capsule was transferred to a 250 mL glass bottle. The extraction solvent containing 5 mL of 25% ammonia, 25 mL methanol, and mL chloroform was added and put into the magnetic stirrer

apparatus (Dragon, Korea) for 10 minutes, and then the bottle was transferred to an ambient place for 30 minutes. The solvent was filtered through filter paper (10 microns). To evaporate the solvents, the filtered solution was connected to a rotary device in a 200 mL balloon. The precipitate was dissolved in 25 mL of chloroform and 10 mL of sulfuric acid 1N using an ultrasonic device. The above solution was transferred to a decanter, and after separating the two phases, the lower phase (chloroform) was removed, and the upper phase (blue color) was kept. The pH of the solution was adjusted between 10 and 11 using a few drops of ammonia solution.

Measurement of morphine concentration in capsules using HPLC

The concentration of morphine was measured by HPLC (Shimadzu, Japan) using a mobile phase of 100 mM ammonium acetate (pH 5.6), dioxane, and acetonitrile at a 1:1 ratio, with a flow rate of 2 mL/min. A 20 µL volume of the extracted solvent was injected into a 250 × 4.9 mm column with 5 µm particles. Detection was performed by measuring absorbance at 250 nm. The standard morphine alkaloid provided by Sigma was used to quantify morphine at concentrations of 2, 5, 10, 20, and 40 ng/mL (Figure S3). The linear regression model was evaluated using analysis of variance, and the correlation coefficient was calculated (Table S1).

Statistical analysis

The statistical analysis used in this study was a Completely Randomized Design (CRD) with three replications. The treatment was at different levels of drought stress, and the traits were the measurement of gene expression and related morphine content changes in the poppy capsule cells. Data were analyzed with one-way ANOVA and Duncan's test for multiple comparisons using SAS v.9.1 software. Data are presented as mean ± SEM. A p-value less than 0.05 compared with the control was considered significant.

Results

RNA integrity and specificity of the RT-qPCR product

The integrity of the extracted RNA from capsule cells was proved by electrophoresis of RNA on agarose TBE gel (Figure 1A). Also, electrophoresis of the qPCR product showed the primer specificity and the absence of primer dimers as well as non-specific PCR products in the process of amplifying the three *T6ODM*, *COR*, and *CODM* genes (Figure 1B).

Journal of Agricultural Science and Technology (JAST), 29(2)
In Press, Pre-Proof Version

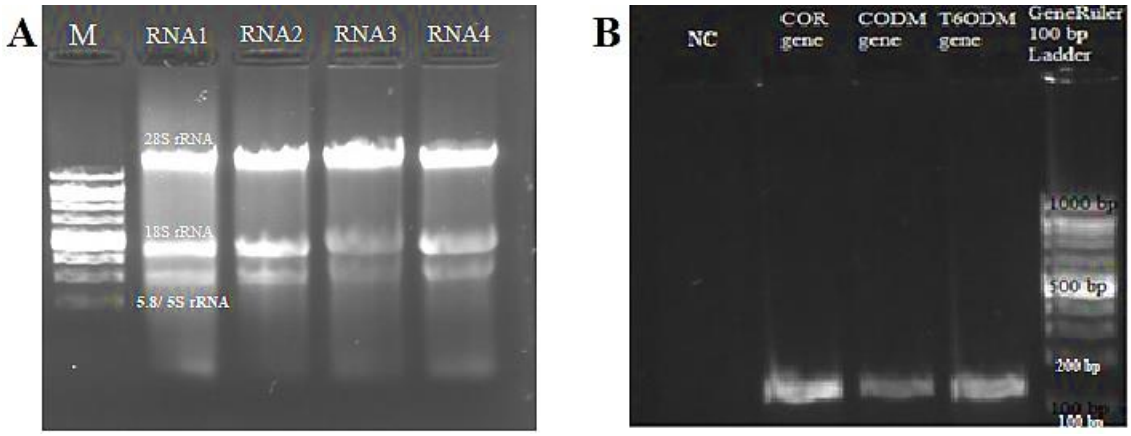

Figure 1. Electrophoresis pattern: A) RNA integrity of capsule cells from different levels and drought stress on TBE agarose gel (1% W/V). B) qPCR products of the *T6ODM*, *COR*, and *CODM* genes. M= GeneRuler 100bp Ladder, NC= negative control.

Effect of different levels of drought stress on gene expression

The statistical analysis showed that the drought stress treatment had a significant effect on T6ODM gene expression in capsule cells ($p< 0.01$) (Table S2). The transcript level of the T6ODM gene was significantly reduced at 80% FC and 60% FC compared with the control plant (FC100%) ($p< 0.01$). Meanwhile, expression of this gene was significantly elevated at FC 40% and FC 20% compared with the control plant (FC100%) ($p< 0.01$). The highest relative expression rate of this gene was 8.46-fold at FC40%, and the fold change of this gene expression was 4.83-fold at FC20% compared to the control plant (Figure 2A). The fold change of the COR and CODM gene expression in capsule cells was less than one in all studied FCs, indicating a significant decrease compared with the control plants (FC100%) ($p< 0.01$). The maximum and minimum reductions in COR gene expression were at 20% FC (0.006) and 40% FC (0.32), respectively, and for CODM gene expression were at 20% FC (0.001) and 40% FC (0.47), respectively (Figure 2B and Figure 2C).

Journal of Agricultural Science and Technology (JAST), 29(2)
In Press, Pre-Proof Version

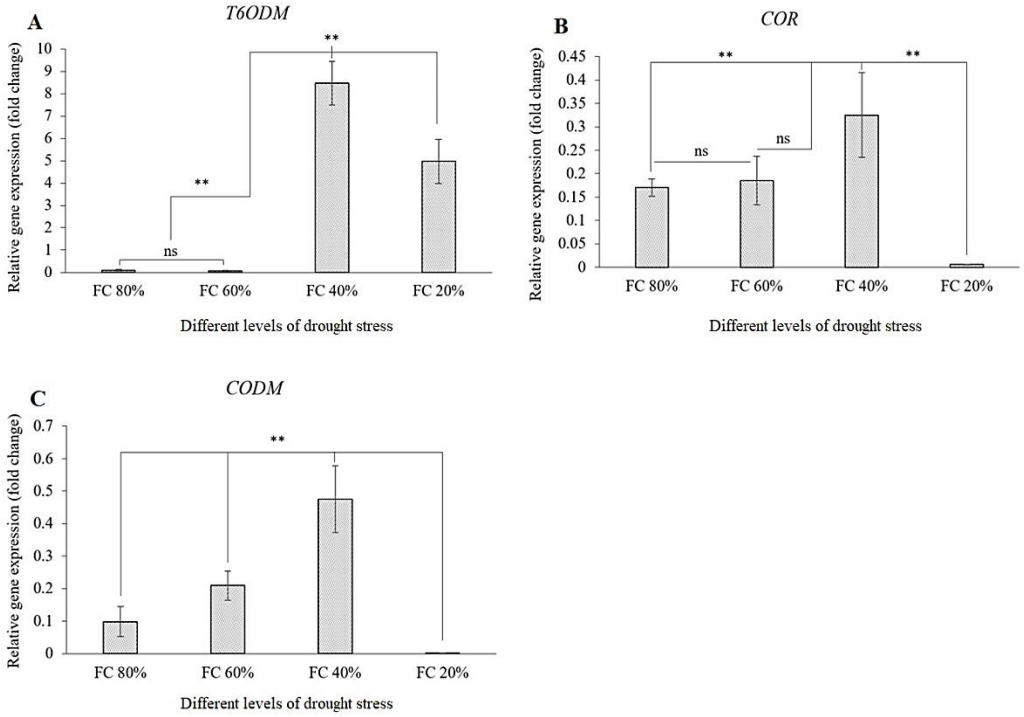

Figure 2. Relative fold change expression of three important genes, including *T6ODM* (A), *COR* (B), and *CODM* (C), involved in the morphine production pathway in capsule cells of poppy under different levels of drought stress. Values are shown as mean ± SEM; n = 6. Data were analyzed with one-way ANOVA and Duncan's test for multiple comparisons. A p-value less than 0.05 compared to the control was considered significant. FC%= Field capacity percent.

Morphine concentration of capsule cells under drought stress

The retention time of the standard morphine peak was 6.22 minutes, according to the results of the HPLC analysis (Figure 3). One-way ANOVA of capsule morphine content revealed that different levels of drought stress significantly changed morphine concentration ($p< 0.01$) (Table S3). The maximum and minimum morphine content were observed at FC40% (1.92 ng/g) and at FC100% and FC80% (0.62 ng/g), respectively (Table 2, Figure 4). Pearson correlation analysis also showed a significant correlation between capsule morphine content and the expression of the T6ODM gene in this tissue ($r =0.99$, $p< 0.01$) (Table 3). The highest correlation among the mRNA concentrations of the three genes (*T6ODM*, *CODM*, and *COR*) was observed between *CODM* and *COR* (r=0.90), but it was not significant ($p>0.05$) (Table 3).

Journal of Agricultural Science and Technology (JAST), 29(2)
In Press, Pre-Proof Version

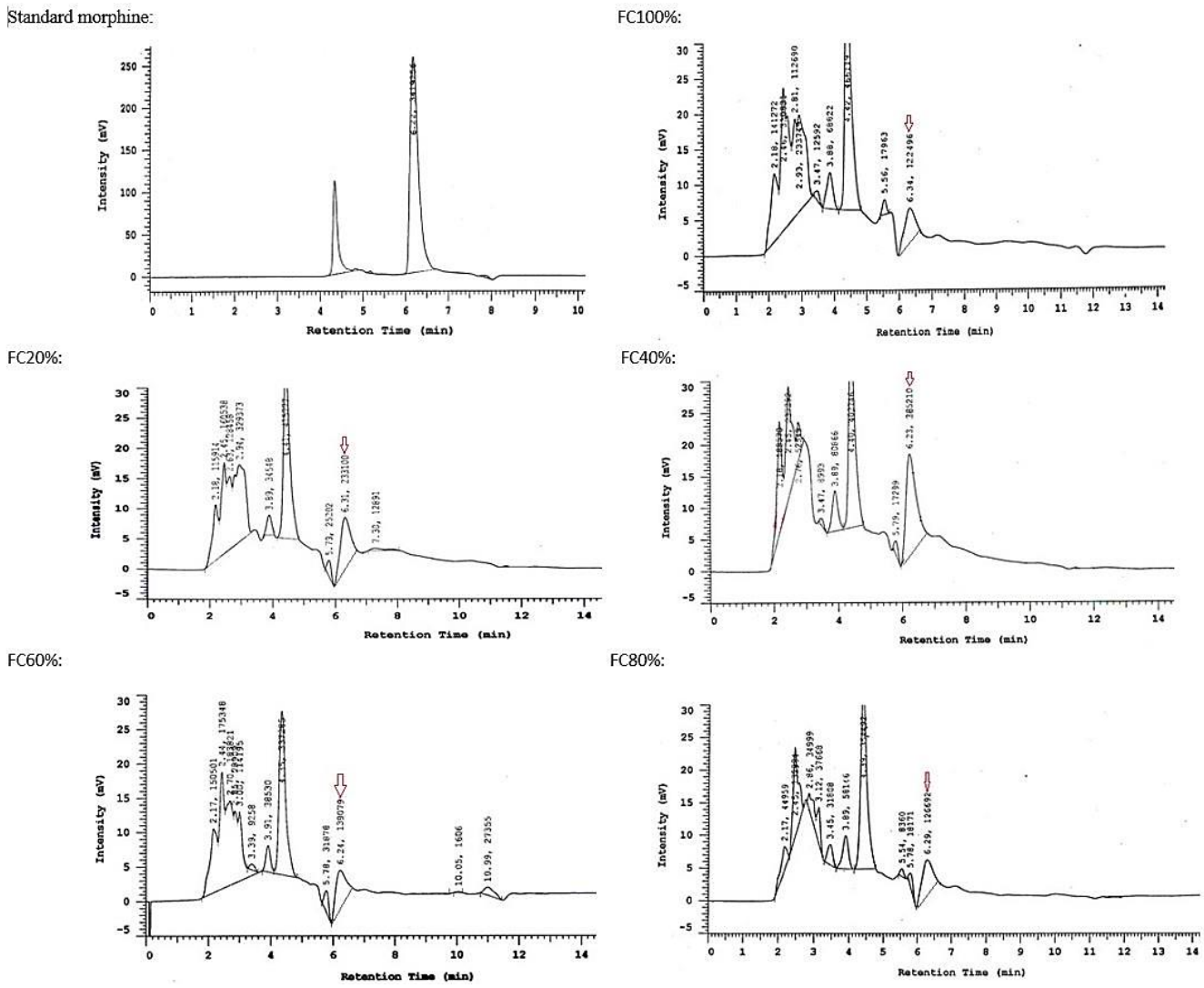

Figure 3. Chromatogram of morphine concentration in capsule cells under different levels of drought stress. The red arrow indicates the peak of morphine. FC%= Field capacity percent.

Table 2. The range of morphine concentration (ng/g) of poppy capsule cells at different FC conditions.

FC percent	Morphine content in capsule cells [a] (ng/g)	Ratio of morphine concentration
100%	0.62	1
80%	0.62	1
60%	0.68	1.1
40%	1.92	3.1
20%	1.17	1.9

a. Mean of 3 replicates

Journal of Agricultural Science and Technology (JAST), 29(2)
In Press, Pre-Proof Version

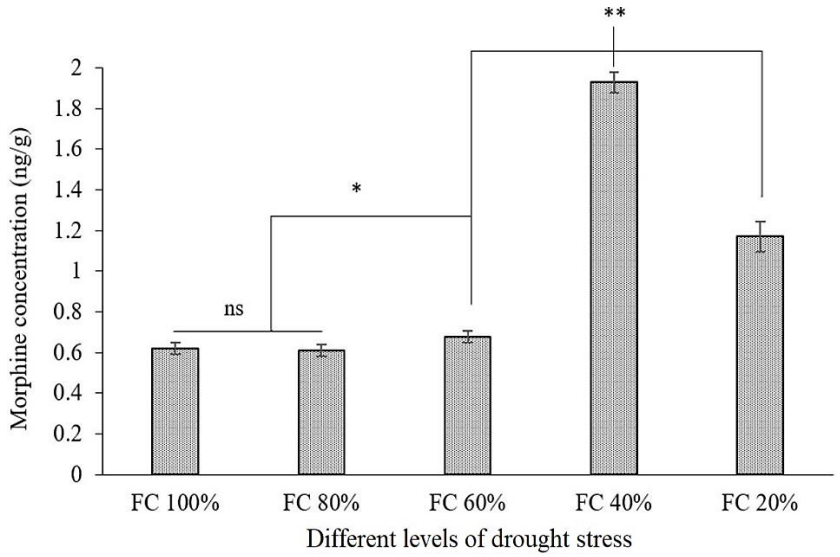

Figure 4. Morphine concentration in capsule cells under different levels of drought stress. Values are shown as mean ± SEM; n = 3. Data were analyzed with one-way ANOVA and Duncan's test for multiple comparisons. A p-value less than 0.05 compared to the control was considered significant. FC%= Field capacity percent. FC%= Field capacity percent.

Table 3. Pearson Correlation Coefficients analysis between morphine content and the expression of three genes (*T6ODM*, *COR*, and *CODM)* under different levels of drought stress in capsule cells of the poppy plant.

	Morphine fold changes	*CODM* fold changes	*COR* fold changes	*T6ODM* fold changes
Morphine fold changes	1	0.64258 (0.3574)	0.52880 (0.4712)	0.99040 (0.0096**)
CODM fold changes	0.64258 (0.3574)	1	0.90227 (0.0977)	0.53129 (0.4687)
COR fold changes	0.52880 (0.4712)	0.90227 (0.0977)	1	0.41582 (0.5842)
T6ODM fold changes	0.99040 (0.0096)	0.53129 (0.4687)	0.41582 (0.5842)	1

Discussion

The three important genes of the morphine production pathway are discussed in this study, as well as their role in morphine production under stress conditions. Many efforts have been made in recent years to identify and improve secondary metabolites such as morphine because of their medicinal properties (Sharma et al., 2013; Mora-Pale et al., 2014). Furthermore, because benzylisoquinoline alkaloids are toxic, their accumulation is limited to specific cells called lattice cells that are adjacent to sieve elements (Le Flem-Bonhomme et al., 2004; Liscombe and Facchini, 2008).

Because benzylisoquinoline alkaloids are produced in specific cells (Chandra and Chandra 2011), abiotic stresses can be used as a practical method to increase the expression of genes that produce these alkaloids. Secondary metabolites play an important role in plants' adaptation to environmental conditions, and a variety of factors trigger their accumulation. Drought, salinity, and cold are all environmental factors that influence plant growth and metabolite production (Akula and Ravishankar, 2011). Drought has also been shown to increase the expression of many plant genes (Abdollahi Mandoulakani et al., 2017; Tang et al., 2012). Drought has also been shown to cause secondary metabolites to be produced in the poppy plants (Szabo et al., 2003). The *T6ODM*, *COR*, and *CODM* genes, which play a key role in morphine biosynthesis, have been discovered in the poppy plant (Beaudoin and Facchini, 2014).

The acceleration of thebaine and codeine conversion into morphine is facilitated by elevated gene expression of *T6ODM* and *CODM*, respectively. Additionally, enhanced expression of the *COR* gene may result in augmented accumulation of codeine and morphine (Allen et al., 2007; Larkin et al., 2007). Consequently, the present investigation aims to elucidate the expression variations of the aforementioned genes across diverse field capacities (FCs) under conditions of drought stress, as well as to explore their association with morphine biosynthesis in the capsule cells of Papaver somniferum.

In the current investigation, drought stress across varying FC levels exhibited a notable reduction in the expression of the *COR* and *CODM* genes; conversely, drought stress at 20% and 40% FC stimulated an increase in *T6ODM* gene expression. Recently, Özcan et al. (2025) reported analogous findings through the application of virus-induced gene silencing (VIGS) in opium poppy. They illustrated that the knockdown of the *CODM* gene resulted in a 107% upregulation of *T6ODM* gene expression. Importantly, the augmentation of *COR* gene expression was associated with a suppression of *T6ODM* gene expression (a 47% reduction in *T6ODM*) and an enhancement of *CODM* gene expression (a 49% increase in CODM). However, they did not assess any correlation between gene expression and morphine concentration (Özcan et al., 2025). It is noteworthy that we monitored the expression of the *T6ODM* gene alongside corresponding morphine concentrations across different FC percentages, revealing that drought stress leads to an increase in both *T6ODM* gene expression and morphine concentration at 40% FC, followed by a decline at 20% FC. Therefore, the synergistic effect of *T6ODM* gene overexpression and drought

stress may be leveraged to enhance morphine alkaloid levels in the breeding programs for the poppy plant.

The concentrations of alkaloids derived from poppy appear to be influenced by various stress factors (Szabo et al., 2003). Drought stress in poppy plants has been shown to elevate alkaloid content, yielding significantly increased levels of morphine, codeine, and narcotine, in accordance with the findings of Szabo et al. (2003), which align with the observations made in this study. Furthermore, drought stress has been correlated with enhanced production of secondary metabolites in poppy plants (Akula and Ravishanka, 2011). As reported by Kaickar et al. (1978), sufficient precipitation before flowering, followed by arid and elevated temperature conditions, is conducive to increased morphine concentrations within the capsule.

Conclusions

In this investigation, the morphine content exhibited a significant increase of 3.1-fold in the capsule cells under 40% FC conditions relative to the control specimens. Subsequently, this content decreased to 1.9, 1.1, and 1-fold under 20%, 60%, and 80% FCs, respectively, compared with the control plants. The findings of this study reveal a significant correlation between the expression of the *T6ODM* gene and the levels of morphine alkaloids in the capsule cells subjected to drought stress treatment; however, such a correlation was not observed for the *COR* and *CODM* genes. Based on the results of this study, it is recommended to pursue the overexpression of the *T6ODM* gene in the poppy plant and to assess the morphine concentration in the capsules for the purpose of plant breeding.

Acknowledgment

The work was supported by a grant from Gorgan University of Agricultural Sciences.

References

1. Akula R, and Ravishankar GA (2011) “Influence of abiotic stress signals on secondary metabolites in plants”. Plant Signal Behav 6: 1720-1731.
2. Allen RS, Miller JAC, Chitty JA, Fist AJ, Gerlach, WL, Larkin PJ (2007) Metabolic engineering of morphinan alkaloids by overexpression and RNAi suppression of salutaridinol 7-O-acetyltransferase in opium poppy. Plant Biotechnol J 6:22–30.

3. Azizyan R, Mandoulakani BA (2024) Partial coding sequence identification, gene expression analysis, and content of anticancer phenolic compounds in Sonchus arvensis L. under drought stress conditions. Ind Crop Prod 209:118030.
4. Bagheri N, Tarinejad A, Hasanpur K, Majidi M (2025) Decoding the Biosynthetic Pathway of the Alkaloid Morphine with Bioinformatics. Agrotechniques in Industrial Crops. 5(4):228-39.
5. Beaudoin GAW, Facchini PJ (2014) Benzylisoquinoline alkaloid biosynthesis in opium poppy. Planta. 240:19–32.
6. Bhardwaj K, Bhargav R, Patocka J, Sharma R, Navratilova Z, Oleksak P, Kuca K (2024) Dendrobine: a neuroprotective sesquiterpenic alkaloid for the prevention and treatment of diseases: a review. Mini Rev. Med. Chem. 24: 1395–1408.
7. Cao Y, Yang W, Ma J, Cheng Z, Zhang X, Liu X, Wu X, Zhang J (2024). An integrated framework for drought stress in plants. Int. J. Mol. Sci. 25.
8. Chandra S, Chandra R (2011) Engineering secondary metabolite production in hairy roots. Phytochem Rev 10, 371–395.
9. Davoudnia B, Ahmadi J, Fabriki-Ourang S (2016) Investigation in expression of key genes involved in sanguinarine biosynthetic pathway in Papaver genus under drought and salinity. Iranian Journal of Genetics and Plant Breeding. 5(2):48-57.
10. Dewick PM (2002) Medicinal natural products: a biosynthetic approach, 2nd. Wiley, Chichester.
11. Duda-Madej A, Viscardi S, Szewczyk W, Topola E (2024) Natural alkaloids in cancer therapy: berberine, sanguinarine and chelerythrine against colorectal and gastric cancer. Int. J. Mol. Sci. 25.
12. Facchini PJ, DeLuca V (1995) Phloem-specific expression of tyrosine/dopa decarboxylase and isoquinoline alkaloid biosynthesis in opium poppy. Plant Cell 7:1811 - 1821.
13. Facchini PJ (2001) Alkaloid biosynthesis in plants: biochemistry, cell biology, molecular regulation and metabolic engineering applications. Annu Rev Plant Physiol 52: 29-66.
14. Facchini PJ, Hagel JM, Liscombe DK, et al. (2007) Opium poppy: blueprint for an alkaloid factory. Phytochem Rev 6: 97–124.
15. Feng K, Li X, Bai Y, Zhang D, Tian L (2024). Mechanisms of cancer cell death induction by triptolide: a comprehensive overview. Heliyon 10, e24335.

16. Frick S, Chitty J A, Kramell R, Schmidt J, Allen R S, Larkin P L and Kutchan T M (2004) Transformation of opium poppy (*Papaver somniferum* L.) with antisense berberine bridge enzyme gene (anti-bbe) via somatic embryogenesis results in an altered ratio of alkaloids in latex but not in roots. Transgenic Res 13:607 – 13.
17. Guedes JG, Ribeiro R, Carqueijeiro I, Guimaraes AL, Bispo C, Archer J, Azevedo H, Fonseca NA, Sottomayor M (2024) The leaf idioblastome of the medicinal plant Catharanthus roseus is associated with stress resistance and alkaloid metabolism. J Exp Bot 75: 274–299.
18. Guo L, Winzer T, Yang X, Li Y, Ning Z, He Z, Teodor R, Lu Y, Bowser TA, Graham IA, Ye K (2018) The opium poppy genome and morphinan production. Science. 362(6412):343-7.
19. Hagel JM, Facchini PJ (2010) Dioxygenases catalyze the O-demethylation steps of morphine biosynthesis in opium poppy. Nat Chem Biol 6:273–275.
20. Hashemi SM, Naghavi MR (2015) Production and gene expression of morphinan alkaloids in hairy root culture of *Papaver orientale* L. using abiotic elicitors. Plant Cell Tiss Org Culture 125:31-41.
21. Jacobs DI, Snoeijer W, Hallard D, Verpoorte R (2004) The Catharanthus alkaloids: pharmacognosy and biotechnology. Curr Med Chem 11: 607–628.
22. Jaleel CA, Gopi R, Sankar B, Gomathinayagam M, Panneerselvam R (2008) Differential responses in water use efficiency in two varieties of Catharanthus roseus under drought stress. CR Biol 331:42-47.
23. Kaicker US, Saini HC, Singh HP, Choudhury B (1978) Environmental effects on morphine content in opium poppy (*Papaver somniferum* L.). Bull Narcotics 3:69–74.
24. Larkin PJ, Miller JAC, Allen RS, Chitty JA, Gerlach WL, Frick S, Kutchan TM, Fist AJ (2007) Increasing morphinan alkaloid production by over-expressing codeinone reductase in transgenic *Papaver somniferum*. Plant Biotechnol J 5:26–37.
25. Le Flem-Bonhomme V, Laurain-Mattar D, Fliniaux MA (2004) Hairy root induction of *Papaver somniferum* var. album, a difficult-totransform plant, by A. rhizogenes LBA 9402. Planta 218: 890–893.
26. Liscombe DK, Facchini PJ (2008) Evolutionary and cellular webs in benzylisoquinoline alkaloid biosynthesis. Curr Opin Biotechnol 19:173–180.

27. Li P, Yan MX, Liu P, Yang DJ, He ZK, Gao Y, Jiang Y, Kong Y, Zhong X, Wu S, Yang J, Wang HX, Huang YB, Wang L, Chen XY, Hu YH, Zhao Q, Xu P (2024a) Multiomics analyses of two Leonurus species illuminate leonurine biosynthesis and its evolution. Mol. Plant 17: 158–177.

28. Li Z, Wang Y, Xu M, Liu H, Li L, Xu D (2023) Molecular mechanism overview of metabolite biosynthesis in medicinal plants. Plant Physiol Biochem 204:108125.

29. Mandoulakani BA, Eyvazpour E, Ghadimzadeh M (2017) The effect of drought stress on the expression of key genes involved in the biosynthesis of phenylpropanoids and essential oil components in basil (Ocimum basilicum L.). Phytochemistry. 139:1-7.

30. Mora-Pale M, Sanchez-Rodriguez SP, Linhard RJ, Dordick JS, Koffas MA (2014) Biochemical strategies for enhancing the in vivo production of natural products with pharmaceutical potential. Curr Opin Biotechnol 25:86–94.

31. Morimoto S, Suemori K, Moriwaki J, Taura F, Tanaka H, Aso M, Tanaka M, Suemune H, Shimohigashi Y and Yukihiro Shoyama Y (2001) Morphine Metabolism in the Opium Poppy and Its Possible Physiological Function. J Biol Chem 276: 38179–38184.

32. Özcan DE, Köm D, Doğan O, Erişen S, Onarici S (2025) Modification of Gene Expression Involved in Alkaloid Production in Opium Poppy by VIGS Combined with Pretreatment of Macerozyme Enzyme. Plant Direct 9(1), e70034.

33. Palesh H, Abdollahi Mandoulakani B (2020) The effect of drought stress on the expression of some genes involved in monoterpene and sesquiterpanes biosynthesis and essential oil compounds in basil. J Med Plants. 19:204-12.

34. Pereira AG, Cassani L, Garcia-Oliveira P, Otero P, Mansoor S, Echave J, Xiao J, Simal-Gándara J, Prieto MA (2023) Plant alkaloids: production, extraction, and potential therapeutic properties. In Natural secondary metabolites: from nature, through science, to industry Feb 22 (pp. 157-200). Cham: Springer International Publishing.

35. Sharma P, Padh H, Shrivastava N (2013) Hairy root cultures: a suitable biological system for studying secondary metabolic pathways in plants. Eng Life Sci 13:62–75.

36. Shukla S, and Singh SP (2000) Alkaloid profile in relation to different developmental stages of *Papaver somniferum* L. Phyton-Horn 41:87-96.

37. Sun Y, and Fernie AR (2024). Plant secondary metabolism in a fluctuating world: climate change perspectives. Trends Plant Sci 29, 560–571.

38. Szabo B, Tyihak E, Szabo LGY, Botz L (2003) Mycotoxin and drought stress-induced change of alkaloid content of *papaver somniferum* plantlets. Acta Botanica Hungarica 45:409–417.

39. Tang Y, Liu M, Gao S, Zhang Z, Zhao X, Zhao C, Zhang F, Chen X (2012) Molecular characterization of novel TaNAC genes in wheat and overexpression of TaNAC2a confers drought tolerance in tobacco. Physiol Plantarum 144:210-224.

40. Unterlinner B, Lenz R, Kutchan TM (1999) Molecular cloning and functional expression of codeinone reductase: the penultimate enzyme in morphine biosynthesis in the opium poppy *Papaver somniferum*. Plant J 18:465–745.

41. Zhang ZJ, Jiang S, Zhao QS (2024) The chemistry and biology of lycopodium alkaloids. Chem. Biodivers. 21, e202400954.

تاثیر تنش خشکی بر روی بیوسنتز مورفین در خشخاش (*Papaver somniferum*)

احد یامچی، علی اصغر نصراله نژاد قمی، شکوه همتی، و سیده ساناز رمضانپور

چکیده

پاپاور سامنیفروم گیاه دارویی مهم که منبع قوی از آلکالوئیدهای بنزیل ایزوکوئینولین مهم دارویی (BIAs) شامل مخدر مسکن کدئین، مورفین، بربرین، سنگوئینارین و پاپاورین می‌باشد. بیان سه ژن کلیدی مسیر تولید مورفین (*T6ODM*، *COR* و *CODM*) تحت سطوح مختلف تنش خشکی مورد بررسی قرار گرفت. بذور خشخاش در شرایط گلخانه کشت شد و سه روز پس از باز شدن گل‌ها، تیمار خشکی اعمال شد. نمونه گیری از کپسول گیاه تحت شرایط تنش خشکی بر اساس ظرفیت های مختلف مزرعه ای (FC)، شامل ظرفیت مزرعه 100 درصد (بدون تنش و به عنوان نمونه کنترل)، 80 درصد ظرفیت مزرعه ، 60 درصد ظرفیت مزرعه ، 40 درصد ظرفیت مزرعه و 20 درصد ظرفیت مزرعه انجام گرفت. آزمایش در قالب طرح آماری کامل تصادفی در سه تکرار انجام شد. تیمار آزمایش سطوح مختلف تنش خشکی و صفات اندازه گیری شده شامل تغییر بیان ژنها و تغییر محتوی مورفین سلولهای کپسول گیاه خشخاش بود. استخراج RNA از نمونه ها و سنتز cDNA انجام شد. بیان ژن *T6ODM* در سلولهای کپسول در سطح تنش خشکی 40 درصد ظرفیت مزرعه نسبت به نمونه کنترل (بدون تنش و به عنوان نمونه کنترل)، افزایش معنی داری داشت ($P< 0.01$). برعکس آن، بیان ژن‌های *COR* و *CODM* در نمونه کپسول در همه‌ی سطوح تنش ظرفیت مزرعه ای کاهش معنی داری یافت ($P< 0.01$). روند تغییرات غلظت مورفین درسلولهای کپسول تحت شرایط سطوح مختلف تنش خشکی ظرفیت مزرعه ای، مطابق با تغییرات بیان ژن *T6ODM* بود بطوریکه میزان غلظت مورفین در سطح تنش خشکی 40 درصد ظرفیت مزرعه نسبت به نمونه کنترل افزایش معنی داری داشت ($P< 0.01$). همچنین، ضریب همبستگی پیرسون رابطه معنی داری بین محتوی موفین و بیان ژن *T6ODM* را در سلول های کپسول نشان داد ($P< 0.01$).