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Estimation of *FLS* Gene Expression in Ginger, Grapes and Date Palms using *Artemisia annua* as a Control Sample

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ABSTRACT

In the current study, the gene expression of *FLS* gene prefixes was studied in the leaves of the plants of *Zingiber officinale, Vitis vinifera, Phoenix dactylifera*. This was done using a sample of *Artemisia annua* leaves as a control sample and using the reference gene 18s rRNA. Relative Quantification gene expression was relied upon as an approved method to extract results. This gene is responsible for encoding Flavonol synthase, which encodes Flavonol compounds in the flavonoid metabolism chain in various plant kingdom plants that perform multiple plant functions as well as directly related to human health. Ginger *Z. officinale* was proved to have the highest gene expression at CT= 31.6, while *V. vinifera* and date palm *P. dactylifera* followed respectively with values 33.78 and 38.31.

Keywords- *FLS, Z. officinale, V. vinifera, P. dactylifera*.

I. INTRODUCTION

The importance of the *FLS* gene lies in encoding Flavonol Synthase, which is one of the most important enzymes involved in the biosynthesis of flavonoids in the plant kingdom (1). FLS enzyme produces Flavonol compounds such as kaempferol, Quercetin, Myricitrin and other compounds by stimulating the exaction of Dihydroflavonols (2). The process of regulating the gene expression of the *FLS* gene is carried out by the transcription factors of the R2R3-MYB family (3). The *FLS* gene is affected and the concentrations of the materials produced by it change by exposing the plant air parts to direct sunlight and the number of hours of solar exposure (4). It is also affected by a specific extent by the quantities and concentrations of trace elements found in the soil such as nitrogen, phosphorus, and other life stressors (5). The medical importance of Flavonols for humans is that they are mainly antioxidant compounds, in addition to being antiviral, bacterial, immune promotions, and anticancer (6). The importance of Flavonols is at the forefront of their analogues of the main group flavonoids because these compounds contain an active hydroxyl OH group in their chemical structure, which gives them double medical efficacy (7). The 18s rRNA gene is one of the most reliable reference genes, as it is not affected by any factor or emergency that can occur in the plant in different surrounding conditions and age stages and maintains a very constant amount of expression (8).

II. MATERIAL AND METHODOLOGY

Sample Collection

Samples of fresh leaves of plants *Zingiber officinale*, *Vitis vinifera*, *Phoenix dactylifera* and *Artemisia annua* were collected from farms and fields surrounding the city of Tikrit, Iraq.

RNA Extraction

RNA was extracted in the Molecular Biology Laboratory in the Department of Life Sciences of the Faculty of Science - University of Tikrit. This was done by preparing, washing and sterilising plant leaves, and

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then starting the extraction process using Tranzol up plus RNA kit, which is equipped by TRANS® and according to the attached instructions.

cDNA Synthesis

After completing the extraction of RNA, single samples of RNA were converted into double cDNA tapes using Easy Script First -Strand cDNA Synthesis Kit, which is equipped by TRANS® and according to the attached instructions and within the program below:

Table 1: cDNA Synthesis Thermal Program

Step	Time	Temp. C Cycle = 40
Denaturation	15 min	42
Annealing	15 min	42
Extension	5 sec	85

Primers

Primers are designed based on the information mentioned in NCBI National Centre for Biotechnology Information:

Table 2: *FLS* Primers in the plants of study

Primer	sequence	Tm (°c)
Z.o FLS	F 5`-ATGACTAGGCTTGCGAAGGA-3` R 5`-CGGGTGTGAGAAGAATGTCA-3`	60
V.v FLS	F 5`-GGAAGAAGGCAAGAGGATGA-3` R 5`-TTCAACAAGGGAAGGGAAGA-3`	60
P.d FLS	F 5`-CGTACAGCGAGAAGCTGATG-3` R 5`-ACGACCTTCTGGTCCATGTC-3`	60
A.h FLS	F 5`-TTGCGAGGAAGCAAAAACT-3` R 5`-ATTGCCTCTCTGCATTGCTT-3`	60

Table 3: 18s rRNA Primer in the plants of study

Primer	Sequence	Tm (°c)
18srRNA	F 5`-CCACTTATCCTACACCTCTC-3` R 5`-ACTGTCCCTGTCTACTATCC-3`	60

Real- time pcr:

The reaction was performed by the Australian-origin Bio Molecular System and the reaction mixture was (Perfect Star Green qPCR Super Mix), equipped by TRANS® and according to the attached instructions, the total reaction volume was 20 ?? L, and according to the thermal program below:

Table 4: Real-time pcr Thermal Program

Cycle	Time	Temperature	Stage
Holding stage 1	3min	94	Enzyme activation
40	15sec	94	Denaturation
	45sec	60	Annealing
95c°/15se-60/1min-95c°/30se-60c°/15se			Dissociation

Statistical calculations

Folding = $2^{-\Delta\Delta CT}$

$\Delta CT = CT$ (gene) $-$ CT (house keeping gene)

$\Delta\Delta CT = \Delta CT$ Treated $-$ ΔCT Control

III. RESULTS

From the results shown and after comparing with the 18s rRNA reference gene and based on the control sample of *Artemisia annua*, it was found that the highest gene expression ratio of the *FLS* gene was in *Zingiber officinale* compared to *Viitis vinifera* and *Phoenix dactylifera*.

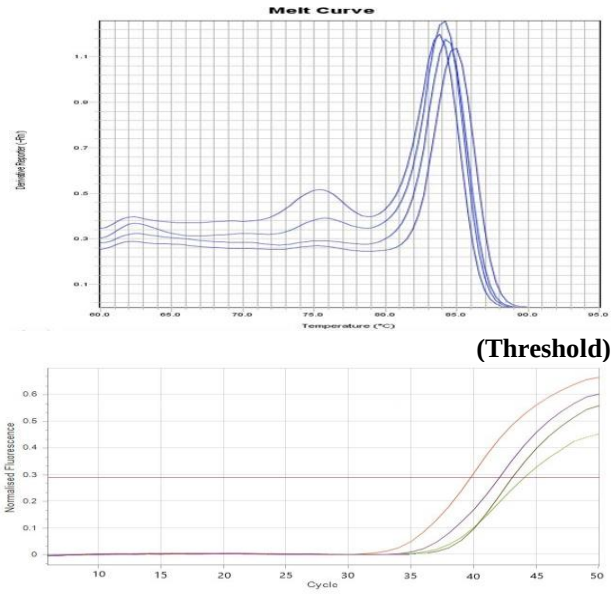

Figure 1: CT threshold value curves and Cycles of the *FLS* gene in Real-time PCR.

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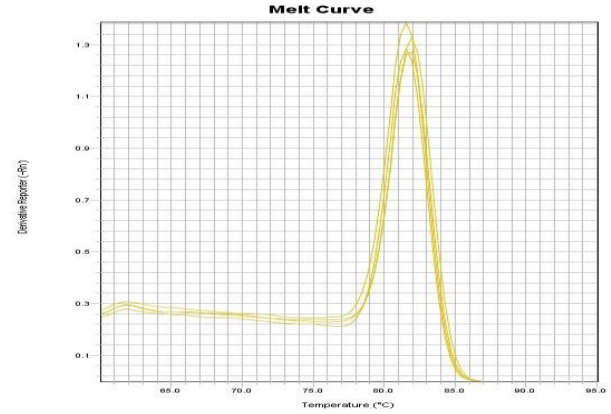
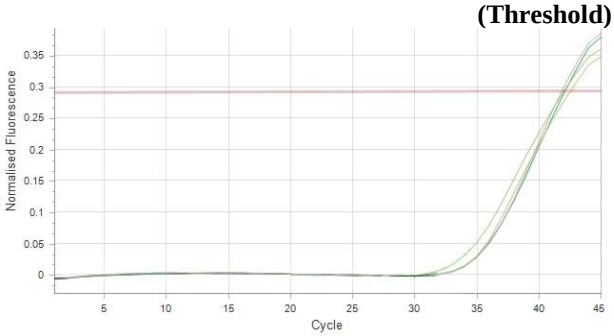


(Threshold)



(cycling)

Figure 2: CT threshold value curves and Cycles of the *18s rRNA* gene in Real-time PCR.

Table 5: CT values for the *FLS* gene

CT	*FLS*
34.23	*Artemisia annua*
31.67	*Zingiber officinale*
38.31	*Phoenix dactylifera*
33.78	*Vitis vinifera*

Table 6: CT values for the *18s Rrna* gene

CT	*18s Rrna*
25.82	*Artemisia herba-alba*
22.72	*Zingiber officinale*
22.89	*Phoenix dactylifera*
25.73	*Vitis vinifera*

Table 7: Shows the value of Folding for the *FLS* gene in the studied samples compared to the control sample

Plant	*18srRNA*	*FLS*	ΔCT	ΔΔCT	Folding
Artemisia	25.82	34.23	8.41	0.0	1.0
Z. officinale	22.72	31.67	8.95	0.54	0.6
P. dactylifera	22.89	38.31	15.42	7.01	7.7
V.vinifera	25.73	33.78	8.05	-0.36	1.2

IV. DISCUSSION

Real-time pcr technology is the best way to study gene expression because of its high sensitivity and high-resolution specificity (10). Gene expression analysis is an important means of detecting and clarifying the mechanism of signaling in plants, as well as clarifying metabolic pathways, developing plant tissues, and the plant's response to external stress (11). It is known in the plant kingdom that flavonoids in general play an "important" role in the process of coloring seeds, flowers and fruits, and that their *TT*-Type genes play an essential role in the process of accumulation of flavonoids in plants in various parts (12). The difference in the genetic compositions of each plant or even at the level of the plant itself is different camel, which belongs to one plant origin (13). The age stage of the plant, the difference in the plant type, the speed, period and duration of germination, the amount of soft and dry weight, the amount of water content, chemical composition, and the environmental circumstance surrounding plants such as humidity, temperature, drought, lack or excess of nutrients, trace elements, number of hours of solar exposure, size and area of leaves, etc. There is an imperative to differ in the expression of the quantity and concentration of secondary metabolites and the expression of the genes responsible for them (14).

V. CONCLUSIONS

From the above, we conclude that the highest percentage of gene expression of the *FLS* gene was in ginger compared to the rest of the study plants, which indicates the inevitability of increasing Flavonol compounds in this plant such as Kaempferol, Quercetin and Myricetin, which are involved in giving it great medical importance due to the importance of these compounds at the medical level and in the field of nutritional supplements in case of increasing clinical examinations and benefitting from this matter, especially in light of the circumstances surrounding us represented by the coronavirus pandemic.

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