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A COMPARATIVE STUDY OF HSP70 GENE EXPRESSION FOR FIVE SPECIES OF *ROSA* L.

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ABSTRACT

This study was aimed to compare and evaluate the increasing or decreasing levels of Hsp70 gene expression of five species of *Rosa* L, 1735 (the most popular ornamental plant that extended throughout the global with high economic value and great cultural importance), *Rosa canina, Rosa damascena, Rosa centifolia, Rosa hybrida* and *Rosa xanthine* through total RNA extraction from plant leaves for the five species then reverse-transcribed to complementary DNA (cDNA) then amplified by RT-qPCR technique as a relative quantitation method .The results demonstrated that gene expression of HSP70 was varied between two seasons, spring and summer seasons. The highest gene expression of HSP70 for the *R. canina* in summer season was recorded at 1.0000 gene fold and 22.950 Ct value as compared with other treated species show low fold of gene expression (0.0012 - 0.0983) gene folding . Our conclusion is that HSP70 elevated levels of gene expression is crucial for heat stress tolerance precisely in *R. canina* enabled to maintain maintain vegetative growth and flowering throughout the year

Key words: RNA extraction, *Rosaceae* , RT-qPCR, *R. canina, R. centifolia , R. damascene*.
***Part of Ph.D. dissertation of the 1st author.**

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INTRODUCTION

Rosa sp. (Family, Rosaceae) is the most popular ornamental plant that extended throughout the global with high economic value and great cultural importance, *Rosa* L. is one of the grand genera of the Rosoideae subfamily (Al-Mathidy et al., 2025). The genus *Rosa* can be divided into three main groups according to their horticultural uses : 1.Rootstock like *R. canina*, 2. Garden Roses like *R. centifolia* and *R. damascena*, 3. Cut Roses like *R. hybrid* and *R. xanthina* . In Iraq, the genus *Rosa* includes: two hybrid species, two subspecies, and four species distributed only in mountain districts (Al-Doskey, 2023); (Vukosavljev, 2014). *R. canina* classified as the only wild type and native in Iraq (Al-Mayah, 2013) ; (Townsend and Guest, 1966). To cope with environmental stress plants have been developed several strategies as a defense mechanisms and resistance to biotic and abiotic stresses (Ismail et al.,2023) ; (Sharma et al., 2023). In plants gene expression experiments required very pure RNA that must be in adequate quantities to build a cDNA library and the extraction technique vary because of the characteristics of different plants (Al-Dallee and Tuama, 2021) ; Jasim et al,2022) . Heat shock proteins (HSPs) have an important role in the refolding and degradation of misfolded proteins that maintain cellular homeostasis hence it have been identified as molecular chaperones (Bourgine and A. Guihur, 2021) (Kotaket al,2007). The Hsps also called (stress-induced proteins) have been classified into five groups based on molecular

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weight (kDa) : (1) Hsp100, (2) Hsp90, (3) Hsp70, (4) Hsp60 and (5) small heat-shock proteins (sHsps) (Al-Whaibi, 2011) ; (Eefoglu, 2009). The Hsp genes are well conserved across organisms and their proteins are expressed in prokaryotic and eukaryotic through evolutionary process (Rosenzweig et al, 2019) ; (Ul Haq et al, 2019). In bacteria Hsps considered as potent activator for the immune system and contribute in biofilm formation (Bajzert and Stefaniak, 2015), while in some pathogenic fungi like *Aspergillus* spp. the Hsps play an important role as virulence factor when subjected to highe temperature to some extent (Al-Barazanchi et al, 2022). In many plants the gene expression of Hsps were studied extensively, (Yang et al, 2014) elucidate that the levels mRNA Hsp70 expression decreased with aging in their study on rotifera and have an important role in anti-aging process, while in another study on two strawberry cultivars were subjected to different levels of heat up to 50 °C, tolerance high temperature stress can be explained with the synergetic effect of Hsp70, Hsp90 and small heat shock proteins (Kesici et al, 2020). The essential role of Hsps in tolerating abiotic stress were investigated in many other plants, in Chinese roses (Jiang et al, 2020), in Sorbus sp. ornamental plant of rosaceae family (Qi et al, 2022), in Arabidopsis (Sung et al, 2001). The genes that encode for HSPs are found and expressed in many organelles of the cell as nucleus, mitochondria, plastids, endoplasmic reticulum and the cytosol and their proteins accumulation also rely on the stress type and intensity (Bourgine and Guihur, 2021) ; (Ul Haq et al, 2019). The aim if this study is to compare the expression of Hsp70 gene through evaluating the increasing or decreasing levels of expression of five species of *Rosa* L. through RNA extraction and qRT-PCR technique

MATERIALS AND METHODS

Fresh leaves with three replicates for each sample were collected during two seasons , spring (April 30℃±2) and summer (heat stress in June 40℃±2) for five species of *Rosa* L., *R. canina*, *R. damascena*, *R. centifolia*, *Rosa hybrida* and *R. xanthine*. The leaves of the cultivated genotypes were cut after making sure that they were pathogens free and cut into small pieces for RNA extraction. Two primers were used in this study the HSP70 (Jiang et al, 2020) and the β-actin as a housekeeping gene (They were synthesized and lyophilized by Alpha DNA Ltd. Canada). (Table 1).

Table 1. Sequences of the Primers of the Study

Gene	Primer	Sequences 5′→3′	reference
HSP70	**F**	**ATGKCVGGAAAGGGAGAGG**	**Jiang et al., 2020 (15)**
	R	**TTARTCAACTTCYTCRATCT**	
β-actin	**F**	**ACCGGTGTTATGGTTGGTATG**	
	R	**CCGTGCTCAATGGGATACTT**	

All samples were subjected for total RNA extraction by using the *TransZol* Up Plus RNA Kit Reagent according to the manufacturer's instructions . The concentration of total RNA ranged from 84-126 ng/ µl., while the total RNA samples purity ranged from 1.95–2.2 ng/µl

Synthesis the cDNA form total RNA

Using the EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix Kit, total RNA was reverse transcribed to complementary DNA (cDNA). According to the manufacturer's instructions, the operation was performed in a reaction volume of 20 µl. (6µl) of total RNA had to be reversely transcribed . HSP70 gene quantitative

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expression were determined by reverse transcription quantitative polymerase chain reaction (RT-qPCR) as a relative quantitation method. The gene expression was normalized to the level of a β-actin gene (housekeeping gene) quantified by the ΔCt value and folding ($2^{-\Delta\Delta ct}$) method (25)

RESULTS AND DISCUSSION

All steps of RT-qPCR were combined with perfect yield of cDNA reflecting efficient reverse transcription. RT-qPCR is characterized by high sensitivity and reliability thus considered a perfect standard for detecting an quantifying gene expression (Aljassani et al, 2022) ; (Al-Salihy et al, 2020) ; (Li et al, 2015) . A reliable fact that the lower Ct value refer to larger amount of starting material (gene copies) (Al-Dallee and Tuama, 2021) ; (Schmittgen and Livak , 2008). Results of the study were recorded as the value of Cycle threshold (Ct) and data presented as "fold change" in gene expression. Detection of HSP70 gene associated with stress tolerance must be convoyed by estimating the percentage of its expression and determining the levels of its decreasing or increasing under consideration genotypes. In terms of gene expression, high Ct values refer to low gene copies and vice versa. The results of Tables (2 and 3) showed significant differences and an increase in the levels of gene expression in mRNA for five *Rosa* species during two seasons . The results showed in (Table 2 and Fig.1) for Ct value and gene folding of HSP70 (target gene) demonstrated the differences of gene expression levels among *Rosa* genotypes .The expression of HSP70 in *R canina* was higher than other genotypes with 22.950 Ct value and 1.0000 fold change during flowering seasons considering it as positive control and *R damascena* was slightly higher with 24.743 Ct value and 0.2258 fold change significant deffirences comparing with the other three genotyps. .The stable exprssion of β-actin was quiet expected as a house keeping gene (Table 2,3 and Fig.2) represented for data normalization.

Table 2. Folding of gene expression of HSP70 in five *Rosa* genotypes Normal Temperature

Genotype	HSP70 Ct	β-actin Ct	ΔCt	ΔΔCt	folding
R. damascena	**24.743**	**23.113**	**1.630**	**0.323**	**0.2258**
R. centifolia	**29.853**	**23.847**	**6.007**	**0.016**	**0.0109**
R. hybrid	**29.283**	**24.360**	**4.923**	**0.033**	**0.0230**
R. xanthina	**28.197**	**23.607**	**4.590**	**0.042**	**0.0290**
***R. canina* (control)**	**22.950**	**23.467**	**-0.517**	**1.431**	**1.0000**

In summer season accompanying with elevation of heat as abiotic stress especially in Iraq many *Rosa* species, *Rosa damascena, Rosa centifolia, Rosa hybrida* and *Rosa xanthine* are failed in vegetative growth and flowering even though will be fragile and burnt shoots (by visual observation of the reasercher) except for *R. canina* that consider as a rootstock and wild species with high tolerance capacity (Al-Doskey , 2023) ; (Maloupa et al, 2021). In (table 3 Fig 1) the values of HSP70 Ct and gene folding were increased as expected in response to elevation of gene expression due to high temperature, however *R. canina* (the treated control) exhibit highest gene folding 1.0000 and 22.950 Ct value compared with other treated genotypes show low fold of gene expression (0.0012 - 0.0983). High temperature stress inhibits the synthesis of normal proteins in plants disturbing cellular homeostasis leading to increases the synthesis of Hsps, failer in flowering of many types of rose was recorded by researchers during heat stress (Jiang et al, 2020) ; (Jiang et al, 2009) ; (Li et al, 2019). Abiotic stresses, epigenetics and other factors

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could modify gene expression (Elsahookie et al, 2021) ; (Qin et al, 2021) demonstrated that Hsps function as molecular chaperones under normal conditions. As expected, the growing environment has a key role in the genetic performance of plants but with different levels due to genetic-environment interaction is investigated (Shenawa and Alfalahi, 2019**).**

Table 3. Folding of gene expression of HSP70 in five *Rosa* genotypes High Temperature

Genotype	HSP70 Ct	β-actin Ct	ΔCt	ΔCt	folding
R. damascene	**26.537**	**23.003**	**3.533**	**0.086**	**0.0012**
R. centifolia	**23.020**	**22.470**	**0.550**	**0.683**	**0.0097**
R. hybrida	**22.853**	**22.830**	**0.023**	**0.984**	**0.0140**
R. xanthina	**20.650**	**23.440**	**-2.790**	**6.916**	**0.0983**
R.canina (control)	**18.223**	**24.360**	**-6.137**	**70.359**	**1.0000**

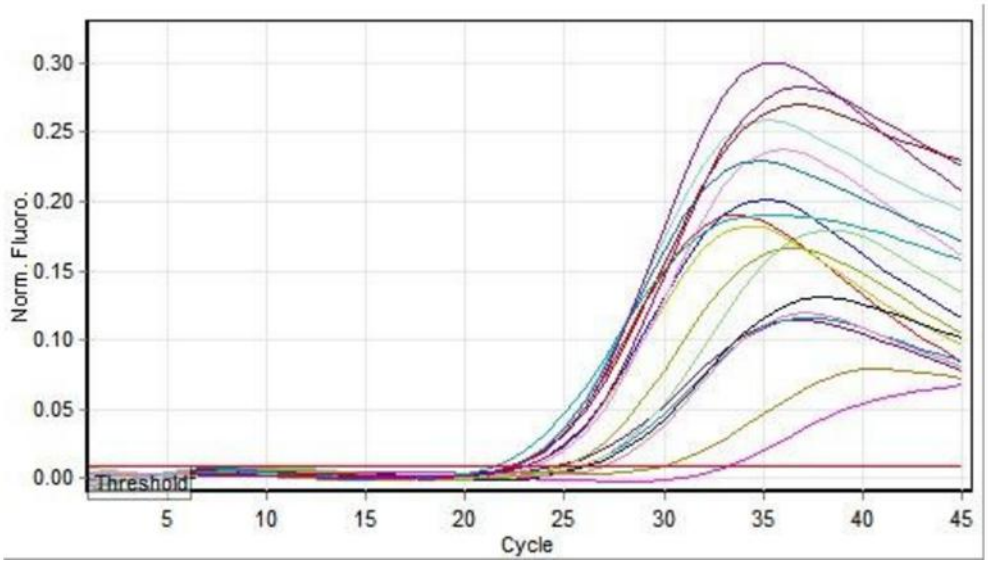

Figure1. A plot representing amplification plot curves of the parameters for the gene HSP70 of *Rosa* genotypes

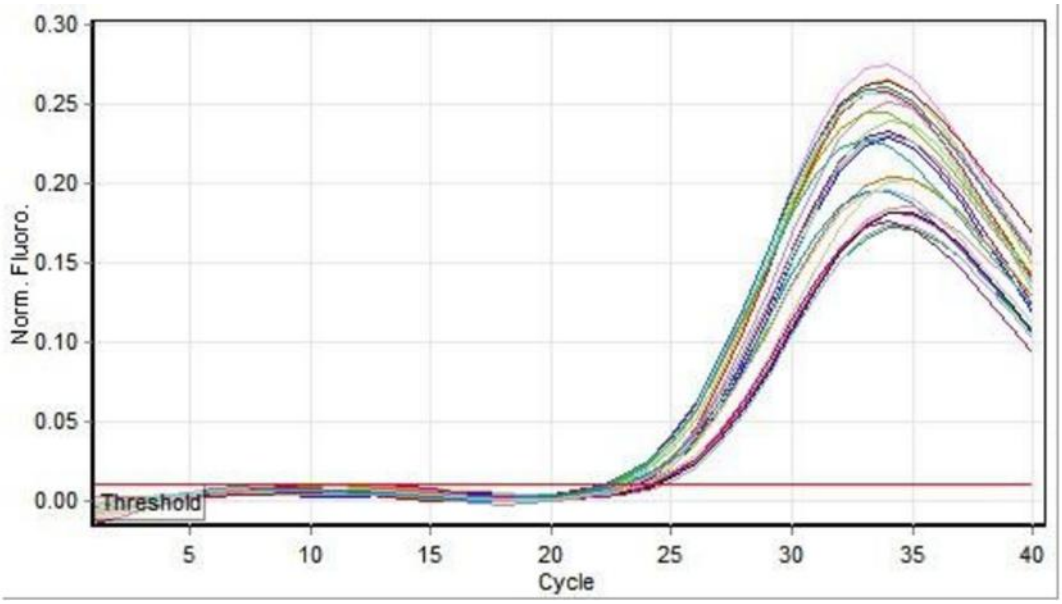

Figure 2. A plot representing amplification plot curves of the parameters for the β-actin (Housekeeping) gene of *Rosa* genotypes

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The correlation between high temperature tolerance and increasing Hsp70 due to their vital role in newly synthesized protein to fold or to protect miss-folded proteins and responsible of disposal and degradation of non- native proteins (Al-Whaibi, 2011) ; (Ul Haq et al, 2019).

CONCLUSION

Inducible HSP70 is expressed in stressed cells with low expression in normal cells, but its level increases rapidly under high temperature precisely in *R. canina* as compare to other species under consideration and that gave this wild type preference over the others in tolerating heat stress and maintain vegetative growth and flowering throughout the year providing an important source for hybridization and to improve new hybrids.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

AUTHORS DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

AUTHOR'S CONTRIBUTION STATEMENT

Bushra M. L, Alwash was responsible for designing the study and editing the manuscript as a supervisor . The experiments were conducted , analyzed and written by Liqaa A. Jazaa as a part of Ph.D. degree study

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دراسة مقارنة للتعبير الجيني للجين HSP70 لخمسة أنواع من نبات الورد *Rosa* spp.

لقاء علي جازع 1 بشرى محمد جاب

قسم علوم الحياة ، كلية العلوم للبنات ، جامعة بغداد

المستخلص

أجريت هذه الدراسة لمقارنة وتحديد التعبير الجيني للجين **HSP70** وتقييم مستويات الزيادة أو النقصان للجين، خمس أنواع من نبات الورد ***Rosa* spp.** تمت دراستها (الذي يعتبر من أهم وأشهر نباتات الزينة المنتشرة حول العالم) ***R. canina, R. damascena, R. centifolia, Rosa hybrida* and *R. xanthine***. تم أستخلاص ال **RNA** من الأوراق النباتية للانواع الخمسة ومن ثم أجري عليه الأستنساخ المعاكس لأنتاج ال **DNA** التكميلي له وبعدها يتم تضخيم النسخ من خلال تقنية **RT–qPCR** كطريقة لقياس النسب الكمية. أظهرت نتائج البحث أن التعبير الجيني للجين **HSP70** أختلفت نسبه لجميع الأنواع خلال الموسمين قيد الدراسة الربيع والصيف حيث زادت مستويات التعبير الجيني، والنوع ***R. canin*** أعطى أعلى القيم لل **ct value** و **gene folding** وكانت **22.950** و **1.0000** على التوالي. نستنتج من هذه الدراسة أن زيادة مستويات التعبير الجيني للجين **HSP70** خصوصا للنوع ***R. canina*** مع أرتفاع درجات الحرارة أعطته ميزة تحمل الظروف البيئية واستمرارية النمو الخضري والتزهير طوال أيام السنة مقرنة مع باقي الأنواع التي لها فترات نمو وتزهير محددة.

الكلمات المفتاحية: أستخلاص **RNA**، العائلة الوردية، الوقت الحقيقي للتضخيم الكمي، ***R. damascena*، *R. canina, R. centifolia***

* البحث مستل من أطروحة دكتوراه للباحث الأول