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DETERMINATION OF GENE EXPRESSION *(WRKY1)* OF SOME TOMATO GENOTYPE UNDER WATER STRESS

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Abstract

Drought is one of the major constraints in plant productivity worldwide and over 30 to 90 years is severe and widespread. Drought scenarios are expected to spread to large parts of the land area. Water stress and low water availability affect both crop and quality crops, reducing crop productivity. The lack of water leads to a lack of nutrient uptake and its direct effect on biological and physiological processes, which is the result of photosynthesis. The aim of the study was to determine the gene expression (WRKY1) of five genotypes from the G5, which chose endurance drought through plant breeding programs (Nossana, Rogina and 1006 and G4) were localized varieties using qPCR The gene expression (WRKY1) responsible for drought tolerance was investigated in genotypes and showed that high levels of stress resulted in increased gene expression, so the highest gene expression values were (27, 857, 9.189, 7.464), respectively The varieties (G55, Nosana, Rogina) are not present in the gene structure (G4,1006) which did not give a high percentage under the same conditions.

Keywords: Water stress, Gene expression, factor WRKY, Genetic structures, Reference genes.

Introduction

Tomatoes *(Solanum lycopersicum*) are fruit trees belonging to the *Solanaceae* family of about 100 genera and 2,500 species, including many of the most important agricultural plants such as potatoes, eggplant, pepper and tobacco (Olmstead *et al*., 2008). The crop, the second most important vegetable crop in the world (Foolad, 2007) is home to South America (Blanca *et al*., 2012). Tomato fruits are used as organic vegetables and are sometimes processed with tomato paste, tomato sauce, tomato juice and ketchup. According to (Mbaka *et al*., 2013), tomato is an economically important horticultural crop in Iraq. The consumption of tomato fruit has gained importance because of its rich antioxidant properties known to reduce cancer incidence (Wamache, 2005), Tomato fruits contain lycopene, carotene, ascorbic acid and phenolic compounds, which have nutritional benefits to consumers One of the main constraints to tomato production is water hypocritical, because the crop is highly sensitive to water shortages that reduce yield and lead to a potential crop failure (Sibomana *et al*., 20130).

Environmental pressures such as cold, salt and drought are important factors affecting the activities of the plant such as high levels of drought, which is a factor limiting the molecular and physiological levels of growth and development of plants and lead to the death of crops (Rhoades and Loveday, 1990). The relative performance of the genotypes under stress and non-stress conditions appears to be a common starting point for the identification of desired genetic sequestration in areas with water scarcity and drought and in low-rainfall areas due to low rainfall or poor distribution during the season (Ahmed and Kadhem, 2017).

Many dehydrating genes are also stimulated by stress and cold, suggesting similar mechanisms for stress responses. These genes are classified into three main groups:

1. Those that encode products that protect plant cells directly from stress such as heat stress proteins (HSPs) or catarrons, LEA proteins, reverse osmosis agents, antifreeze proteins, detoxification enzymes, Free radicals (Bray *et al*., 2000; Wang *et al*., 2000).
2. Those involved in sequencing and control of transcription, such as methane-activated protein kinase (MAPK), calcium-based kinase protein (Ludwig *et al*., 2004) and SOS kinase (Zhu *et al*., 2001) phospholipases (Frank, 2000) Copy factors (Cho *et al*., 2000; Shinozaki *et al*., 2000).
3. Those involved in the absorption and transport of water and ions such as aquaporins and ion transporters (Blumwald *et al*., 2000)

Over the past few years, text analysis has indicated that distinct environmental pressures are triggering similar responses. The overlap between stress responses can explain the phenomenon known as cross-stress, the ability to reduce the collateral damage caused by other stress associated with initial stress. Responses to abiotic pressures require the production of important metabolic proteins, such as those involved in the synthesis of *osmoprotectants* and regulatory proteins that operate in signal transfer pathways, namely kinase or transcription factors (TFs). Regulating these responses requires proteins that work in signaling pathways, such as transcription factors, that regulate gene expression by binding DNA sequences to the target genes involved. This type of proportional regulatory system is called regulation. WRKY, as one of the largest transgenic families in plants, has become one of the leading areas of research on plant defense responses (Chen, 2012 Tripathi, 2014)

Biotechnology techniques are advanced in plants to support diagnostic purposes such as microarray DNA (Kawaura *et al*., 2006) and real time qPCR (Al-Mashhadani *et al*., 2016) qPCR technique was used to study the expression profiles in tomatos.

Material and Method

The experiment was carried out in the Faculty of Agricultural Engineering Sciences / University of Baghdad during the spring season 2018. The experiment included two factors: the use of five genotypes (G1, G2, G3, G4 and G5). The second factor is the use of three stages of irrigation (S1, S2, S3) day. The experiment was carried out according to the nested design. The transactions were distributed according to the design of the complete randomized segments and three

replicates**.** The amount of water added to all experimental units was calculated continuously throughout the experimental period

- Determine the soil moisture content available in the soil by reading the soil model of the dried field (constant weight) and comparing it with the field capacitance, as it is perfumed for the purpose of compensating the depleted moisture according to (Allen *et al*., 1998) $d = (\theta_{fc} - \theta_I) \times D$
- Calculate the coefficient of reduction of irrigated area by relying on the area covered by the plant from the soil surface at each stage of its growth and according to equation. (Keller and Karmeli (1974))

$$K_r = \frac{G_C}{0.85}$$

Gene expression

Extraction of DNA

DNA extraction was performed according to the ZR plant RNA Mini Prep ™ Kit with the extraction kit.

Measurement and purity of DNA

RNA quantification was assessed by testing with Promega/USA Quantas. The concentration of isolated RNA was 200-150 ng and the purity ranged from 2 to 1.7 for every 100 milligrams of plant tissue.

Real-Time PCR (one-step RT-qPCR)

RT-PCR Cycling Program

Step	Temp. (°C)	Time	Cycle	Scanning
Reverse transcription	42 °C	10 min	RNA to cDNA	
Enzyme activation	95 °C	3 min	Hold	
Denaturation	95.0 °C	15 sec	40	
Annealing/Extension	55.0 °C -	15 sec		

Results and Discussion

Table 1: The *WRKY1* gene *&* Reference gene *Beta-actin*

Primer	Sequence	Tm (ºC)	GC (%)
Forward	5'-AGGGTAGTTCGAGTACCGGC - 3'	58.6	60
Reverse	5'-ACGTGCTGGACACCCTCTTA - 3’	58.3	55
Forward	TGG CAC CCG AGG AGC ACC CTG		
Reverse	GCG ACG TAC ATG GCA GGA ACA		

The data obtained from real time experiments were detected according to the Ct values which calculated from cycles and was proportional to the starting target copy number (logarithmic scale) used for amplification (the point that the fluorescence signal increased above baseline is the threshold cycle) which are inversely related to the amount of starting template that mean the high value of Ct refers to the low levels of gene expression or amplification gene, (Inverse relationship) while low Ct value indicate high level of gene expression or high copy of gene amplification.

Amplification plots appeared when the fluorescent signal from sample is plotted against cycle number; however amplification plots include the accumulation of product through the period of qPCR experiment.

The expression of gene was detected successfully by using new molecular technique which is Real time PCR (qRT-PCR) with used specific primer. The amplification accuracy of gene product was noticed by the value of cycle threshold (Ct) for the triplicate reactions.

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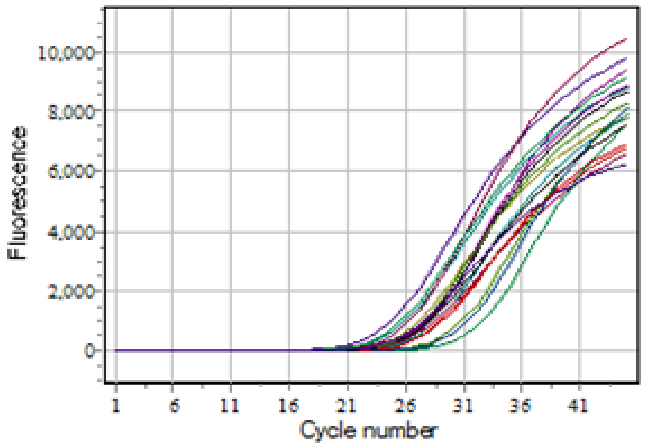

Through the period of qPCR experiment the gene expression of (WARKY1) transcripts significantly increases during drought conditions in both genotypes G5 and G3 Maximum fold expression were noted genotype G5 27.85 under drought stress followed by genotype G3 under drought stress condition. WRKY genes were among several families of transcription factor genes that are well evidenced to have important regulatory roles in plants subjected to various high-salinity or drought stresses (Zou *et al*., 2007). The stress leads to trigger some of the key enzymes of antioxidant defense system. To resist oxidative damage in plants the antioxidant enzymes and certain metabolites; play a vital role leading to adaptation and the ultimate survival under stress (Zhang *et al*., 2007). In the present study, we speculate that the expression of WRKY in turn regulating the expression of other stress related antioxidative genes under drought stress conditions. The expression of antioxidative enzymes enhances the scavenging activity in plants and reduces the ROS produced under stress.

Table 2 : Rate values CT and folding gene WARKY1 in tomato genotype using Real-Time PCR.

Sample	stressed plants		ΔCt	Sample	control		ΔCt	ΔΔCt	folding
	target Ct	Rfer Ct			target Ct	Rfer Ct			
W1G1	25.9	21.9	4	W2G1	28.2	21.3	6.9	-2.9	7.464264
W1G2	25.7	21.7	4	W2G2	27.5	22.6	4.9	-0.9	1.866066
W1G3	29.3	25.9	3.4	W2G3	31.7	25.1	6.6	-3.2	9.189587
W1G4	21.4	25.6	-4.2	W2G4	25	27.7	-2.7	-1.5	2.828427
W1G5	21.2	25.5	-4.3	W2G5	27.2	26.7	0.5	-4.8	27.85762

References

Allen, R.G.; Perreira, L.S.; Raes, D. and Smith, M. (1998). Crop evapotranspiration: Guidelines for computing crop water requirements. Irrigation and Drainage Paper N 56, FAO. Rome, Italy.

Ahmed, M.S and Kadhem, F.A. (2017). Application of multivariate analysis to identify drought tolerance genotype of Maize. The Iraq Journal of Agricultural Sciences, 48(4): 972- 983.

Al-Mashhadani, I.I.D.; Majeed, M.; Ismail, E.N. and Kadhim, M.S. (2016). Detection of salt tolerant gene (TaNIP) and its expression in three selected wheat genotypes through plant breeding programs under salinity conditions, International Journal of Applied Agricultural Sciences, 2: 12-16.

Blanca, J.; Can˜izares, J.; Cordero, L.; Pascual, L.; Diez, M.J. and Nuez, F. (2012). Variation revealed by SNP genotyping and morphology provides insight into the origin of the tomato. PLoS ONE, 7(10), e48198. DOI: 10.1371/Journal. Pone.

Bray, E.A.; Bailey-Serres, J. and Weretilnyk, E. (2000). Responses to abiotic stresses. In: Gruissem, W.; Buchannan, B.; Jones, R. (eds.) Biochemistry and molecular biology of plants. American Society of Plant Physiologists, Rockville, MD, 1158-249.

Blumwald, E. (2000). Sodium transport and salt tolerance in plants. Curr. Opin Cell Biol, 12: 431-434.

Chen, L.; Song, Y.; Li, S. (2012). The role of WRKY transcription factors in plant abiotic stresses. Biochim. Biophys. Acta., 1819(2): 120-128.

Choi H.I.; Hong, J.H.; Ha, J.; Kang, J.Y. and Kim, S.Y. (2000). ABFs, a family of ABA-responsive element binding factors. J. Biol. Chem., 275: 1723-30.

Foolad, M.R. (2007). Genome mapping and molecular breeding of tomato. International Journal of Plant Genomics, 64358.

Frank, W.; Munnik, T.; Kerkmann, K.; Salamini, F. and Bartels, D. (2000). Water deficit triggers.

Keller, J. and Karmeli, D. (1974). Trickle irrigation design parameters of ASAE 17 : 678-685.

Kawaura, K.; Mochida, K.; Yamazaki, Y. andOgihara, Y. (2006). Transcriptome analysis of salinity stress responses in common wheat using a 22 koligo-DNA micro array, Funct. Integr Genomics, 6(2): 132-42.

Ludwig, A.; Romeis, T. and Jones, J.D. (2004). CDPK mediated signalling pathways: specificity and cross-talk. Journal of Experimental Botany, 55: 181-188.

Mbaka, J.N.; Gitonga, J.K.; Gathambari, C.W.; Mwangi, B.G.; Githuka, P. and Mwangi, M. (2013). Identification of knowledge and technology gaps in high tunnels tomato production in Kirinyaga and Embu counties.

Olmstead, R.G.; Bohs, L.; Migid, H.A. and Santiago-Valentin, E. (2008). A molecular phylogeny of the Solanaceae. Journal of Taxonomy 57: 1159-1181.

Rhoades, J.D. and Loveday, J. (1990). Salinity in Irrigated Agriculture. In: Irrigation of Agricultural Crops. Stewart, B.A. and Nielsen, D.R. (ed.)Agron. Monogr. 30. ASA, CSSA and SSSA, Madison, WI.

Sibomana, I.C.; Aguyoh, J.N. and Opiyo, A.M. (2013). Water stress affects growth and yield of container grown tomato (*Lycopersicon esculentum* Mill.) plants. Global Journal of Biochemistry and Biotechnology, 2(4): 461- 466.

Shinozaki, K. and Yamaguchi-Shinozaki, K. (2000). Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signalling pathways. CurrOpin Plant Biol., 3: 217-223.

Saibo, N.J.M.; Lourenco, T. and Oliveira, M.M. (2008). Transcription factors and regulation of photosynthetic and related metabolism under environmental stresses. Annals of Botany, 103: 609–623.

Tripathi, P.; Rabara, R.C.; Rushton, P.J. (2014). A systems biology perspective on the role of WRKY transcription factors in drought responses in plants. Planta. 239(2): 255-566.

Wamache, A. (2005). Vegetable seeds handbook. Regina seeds Seminis. Printed by Bizone ltd. Nairobi Kenya.

Wang, F.; Kang, S.; Du, T.; Li, F. and Qiu, R. (2011). Determination of comprehensive quality index for tomato and its response to different irrigation treatments. Agricultural Water Management Journal 98: 1228- 1238.

Zhu, J.K. (2001). Plant salt tolerance. Trends Plant Sci., 6: 66–67.

Zou, X.L.; Shen, Q.J. and Neuman, D. (2007). An ABA inducible WRKY gene integrates responses of creosote bush (*Larrea tridentata*) to elevated CO_2 and abiotic stresses, Plant Sci., 172: 997–1004.

Zhang, F.Q.; Wang, Y.S.; Lou, Z.P. and Dong, J.D. (2007). Effect of Heavy Metal Stress Anti-oxidative Enzymes and Lipid Peroxidation in Leaves and Roots of Two Mangrove Plant Seedlings (Kandeliacandel and Bruguieragymnorrhiza). Chemosphere, 67: 44-50.