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 (Olmeda 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., 2013).

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 tomatoes.

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) 
  \[ d = (\theta_e - \theta_r) \times D \]
- 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)) 
  \[ K_c = \frac{C_C}{0.85} \]

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

<table>
<thead>
<tr>
<th>Step</th>
<th>Temp. (°C)</th>
<th>Time</th>
<th>Cycle</th>
<th>Scanning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse transcription</td>
<td>42 °C</td>
<td>10 min</td>
<td>RNA to cDNA</td>
<td></td>
</tr>
<tr>
<td>Enzyme activation</td>
<td>95 °C</td>
<td>3 min</td>
<td>Hold</td>
<td></td>
</tr>
<tr>
<td>Denaturation</td>
<td>95.0 °C</td>
<td>15 sec</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Annealing/Extension</td>
<td>55.0 °C - 60 °C</td>
<td>15 sec</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results and Discussion

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

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Tm (°C)</th>
<th>GC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward</td>
<td>5'-AGGGTAGTTCGAGTACCCGGC - 3'</td>
<td>58.6</td>
<td>60</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-ACGTGCTGGACACCCTTA - 3'</td>
<td>58.3</td>
<td>55</td>
</tr>
<tr>
<td>Forward</td>
<td>TGGCACCCAGGAGACCCTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reverse</td>
<td>GCGACGTCATGCGAAGAACA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Through the period of qPCR experiment the gene expression of (WRKY1) 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. In the present study, we speculate that the expression of WRKY in turn regulating the expression of other stress related antioxidant 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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>stressed plants target Ct</th>
<th>Δ Ct</th>
<th>control target Ct</th>
<th>Δ Ct</th>
<th>ΔΔ Ct</th>
<th>folding</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1G1</td>
<td>25.9</td>
<td>4</td>
<td>W2G1</td>
<td>28.2</td>
<td>6.9</td>
<td>-2.9</td>
</tr>
<tr>
<td>W1G2</td>
<td>25.7</td>
<td>4</td>
<td>W2G2</td>
<td>27.5</td>
<td>4.9</td>
<td>-0.9</td>
</tr>
<tr>
<td>W1G3</td>
<td>29.3</td>
<td>3.4</td>
<td>W2G3</td>
<td>31.7</td>
<td>6.6</td>
<td>-3.2</td>
</tr>
<tr>
<td>W1G4</td>
<td>21.4</td>
<td>-4.2</td>
<td>W2G4</td>
<td>25</td>
<td>-2.7</td>
<td>-1.5</td>
</tr>
<tr>
<td>W1G5</td>
<td>21.2</td>
<td>-4.3</td>
<td>W2G5</td>
<td>27.2</td>
<td>0.5</td>
<td>-4.8</td>
</tr>
</tbody>
</table>

References


