

# **CHAPTER 5**

## **SUMMARY AND CONCLUSION**

Wheat (*Triticum aestivum* L.) is the first ranking cereal crop in the globe in terms of area and production. It is thermosensitive long day crop (**Sahu *et al.*, 2002**), the optimum temperature of wheat is 15-18 °C (Choudhury and Wardlaw, 1978) but it grown in this region is exposed to chronic heat stress during certain physiological stages of crop growth. In the 21st century, average surface temperatures of the earth are likely to be increased. Global climate models predict an increase in mean ambient temperatures between 1.8 and 5.8°C (IPCC, 2007). Globally, warming as a result of climate change will affect about seven million hectares of wheat throughout the life cycle and 40% crop faces terminal heat stress potentially increasing food insecurity and poverty (**Tubiello *et al.*, 2000**).

Wheat is predominately produced and consumed in densely populated tropical and subtropical region of the developing world. The local production of wheat in Egypt is about 8 million tons however; it covers less than 60% of local consumption. So, Egypt remains the world's largest wheat importer. Accordingly, cereal import requirements in the current marketing year 2013/14 (July/June) are put at about 6.5 million tonnes, (**FAO 2013**). Analyses of current climatic trends reveal a warming trend in recent decades with country averaged mean temperature increases of 1.4°C and 2.5°C projected by 2050 and 2100. Climate change studies predict a reduction in the productivity of two major crops in Egypt: wheat and maize by 15% and 19% respectively by 2050 (**NEEDS, 2010**).

According to the International Maize and Wheat Improvement Center (**CIMMYT**), climate change may affect wheat production through the direct effects on yield via physiological processes. Plants detect changes in ambient temperature through perturbations in metabolism, membrane fluidity, protein conformation and assembly of the cytoskeleton (**Ruelland and Zachowski, 2010**). Such reactions activate adaptive processes like expression of heat shock proteins, until new cellular equilibriums are reached. However, temperatures above the optimum for growth can be deleterious, causing injury or irreversible damage, which is generally called 'heat stress' (**Wahid *et al.*, 2007**).

To adapt new crop varieties to the future climate, we need to understand how crops respond to elevated temperatures and how tolerance to heat can be improved. At the biochemical and molecular levels the synthesis of heat shock proteins (HSPs) mainly chaperones or proteases play the essential role of preventing or minimizing the deleterious effects of heat at the cellular and molecular levels. Moreover, they help cells in recovering from the stress during the post stress phase (**Larkindale *et al.*, 2005**). This role was confirmed by a study on HSP101. This protein is part of a molecular complex involving also small HSPs (sHSPs), and has the role of resolubilizing protein aggregates formed as an effect o the heat stress (**Agarwal *et al.* 2003**).

The objective of this study was to evaluate heat tolerance of six Egyptian bread wheat varieties (Misr1, Sids12, Misr2, Giza168, sakha93 and Gemmeiza9). Evaluate heat tolerance through physiological and biochemical measurements including cell membrane thermostability, proline content and chlorophyll content.

At a molecular level, develop SNPs marker in *HSP101c* gene of Egyptian bread wheat. Expression analysis using Quantitative (Real-Time) reverse transcription polymerase chain reaction (RT-PCR) used to quantify the relative abundance of HSP101 transcripts under different heat stress conditions. The relevant cDNAs were sequenced and single nucleotide polymorphisms (SNPs) specific for the different polymorphism, were identified

### **Physiology and biochemical study:**

Six most common Egyptian wheat varieties were selected to evaluate heat tolerance, through physiological and molecular approaches. After an initial 8 days growth at 25° C day/night, three sets of plants (control) were maintained at the same conditions, while the other sets of plants was exposed to HT (at 35° C day/night). Effect of heat stress was analyzed after 48 hours (Vegetative).

### **The main results of physiological and biochemical study are summarized in the following points:**

1- Based on membrane thermostability (MTS) test, variety which showed equal or greater than 50% membrane injury was: Misr1, Sids12, Misr2 (82.85, 77.91, 75.246) respectively were considered as heat sensitive (HS) variety. Varieties Sakha93 and Gemmeiza9 showed less than 50% membrane injury in membrane thermostability test (45.93, 48.63) respectively and were grouped as heat tolerant (HT) varieties.

2-Misr 2 showed the most heat stress mediated reduction in total chlorophyll content (87.6%) followed by Misr1, Giza 168 and sid 12 by ( 76.8%, 75.9% and 61.6%) respectively. On the other hand, Gemmeiza9 as a heat tolerant variety which was closely followed by variety sakha93 showed (41 and 53.66) % respectively.

3-The results of present research showed that increasing temperature lead to increase of proline content of ten days seedlings was influenced significantly by the interaction effect of temperature regimes and Egyptian wheat varieties. Results showed that Seedling proline content at 35°C was higher compared to those at 25° C. The highest mean seedlings proline content was observed variety Gemmeiza9 which was closely followed by varieties Giza168, (1.824, 1.747) respectively and The lowest mean value was observed in Misr2 (0.833). At 35° C Giza 168, Sakha 93 and Gemmeiza 9 varieties produced more than double (> 200%) proline than that at 25° C. The highest relative value was observed in variety sakha 93, (246). The relative value proline content higher amount was found in HT variety compared to that in HS variety expected variety Giza168 which was observed as HS variety but showed high relative value proline content.

4-Wheat heat tolerant seedlings revealed a strong positive correlation ( $r = 0.728$ ), ( $r=0.51$ ) and strong negative correlation ( $r = - 0.966$ ) between (relative membrane injury & relative seedling proline content), (relative membrane injury & relative chlorophyll content) and (relative seedling proline content& relative chlorophyll content) respectively.

5-Heat sensitive seedling showed a negative correlation ( $r = 0.214$ ), ( $r = 0.764$ ) and ( $r = 0.352$ ) between (relative membrane injury & relative seedling proline content), (relative seedling proline content& relative chlorophyll content) and (relative membrane injury & relative chlorophyll content).

**In general**, based on Physiological and biochemical studies Misr1, Misr and Sids12 considered as heat tolerant varieties. On other hand, Giza168, Sakha93 and Gemmeiza 9 considered as heat sensitive varieties. Moreover, there is a strong correlation between the increase of proline content and chlorophyll content in relation to relative membrane injury especially under the stress of heat.

## Molecular studies:

Seeds were germinated at 25° C for 14 days as control,(c). Three sets of seedlings were exposed to 34°C for 24 h and then shifted to 42 °C for 2 h (A24+S) post-acclimation and another three stressed sets without acclimation was achieved by exposing plants directly to 42 °C for 2 h (S).

## The main results are summarized in the following points:

### i. Quantitative RT-PCR

- 1- Quantitative RT-PCR revealed that *HSP101* is expressed constitutively in all wheat varieties control and treatment by heat until 14 days after germination.
- 2- At heat treatment (S) variety sakha93 which was closely followed by varieties Misr1 and Gemmeiza9 showed up regulation of *HSP101* expression, (192.7, 143.1, 126.2) % respectively. Variety Giza168 showed lower transcripts of *HSP101* under this heat stress treatment.
- 3- Heat-tolerant Variety Gemmeiza9 which was followed by Variety Misr1 expressed high transcription of *HSP101* gene (429.7, 220.3) % under (A24+S). But, cultivar sids12 show the least *HSP101* transcripts (6.6) % under this treatment.
- 4- The total average of *HSP101* expression of Seedling exposed to adaptation (A24+S) was (181.5%) in comparison to seedling treated by heat directly (s) (113.7%).

**In general**, heat tolerant variety ‘Gemmeiza9’, showed high expression of *HSP101* at two heat treatments (S) and (A24+S). But, heat susceptible variety ‘Sids12’ showed considerable reduction in *HSP101* transcripts at heat treatments (S) and (A24+S). Moreover, Seedling exposed to adaptation (A24+S) induced over expression of *HSP101* by about (59.6%) increment than seedling treated by heat directly (s).

### ii. SNPs detection:

- A. SNPs manually method was obtained by the alignment of the two sequences (*HSP101\_Sids2* and *HSP 101\_Gemmeiza9*) with Anchor sequence *HSP101c* using the DNAMAN® software. *sids12 hsp101*( sensitive variety) sequence revealed 11 transtions from ‘A’ to ‘G’ accounting for 61% of the total mutations, 5 transversions accounting for 28% and two deletions (A) base represented (11%) of the total mutations. The SNP frequency was about one SNP per 112 bp and one InDel per 898 bp. No polymorphism was detected in *Gemmeiza9 HSP101* (tolerant variety) sequence with the exception of two ‘G’ nucleotide deletions of the total mutations.
- B. Detecting SNPs by automated method using SNPs finder database revealed 8 Transtions from (A) to (G), 2 transversions from (A) to (T) and 2 InDels were detected at *HSP101\_Sids12* sequence. The SNPs distribution was: (66%) transitions, (17%) transversions and (17%) InDel. No polymorphism was detected in *Gemmeiza9 HSP101* sequence expected one InDel.

**In general**, it can be noticed that the results of automated method were almost in agreement with the manual accounting especially for the SNPs transtions from ‘A’ to ‘G’ was the most frequent event, accounting for more than 60% of the total *Sids12\_HSP101*

sequence mutations at total length of 1795 bp and No polymorphism was detected in *Gemmeiza9 HSP101* sequence.

### iii. Prediction amino acids sequence polymorphism:

**A.** Alignment of the deduced amino acid sequences of *TaHSP101c*, *Gemmeiza9\_HSP101* and *Sids12\_HSP101* obtained with DNAMAN® software (Lyon BioSoft, Quebec, Canada) showed that *Sids12\_HSP101* shares 97.9% identity with *AF174433.1 (HSP101c)* and *Gemmeiza9 HSP101* share 99.5% identity with *AF174433.1 (HSP101c)*.

### **B. Detection of amino acids changes by Mega 6.06 program revealed:**

1. No effective changes at *Gemmeiza9\_HSP101*, while, there are ten amino acid position changes in *Sids12\_HSP101*.
2. Relative percentag change of amino acids frequencies at sequences of *Sids12\_HSP101* to *AF174433.1 TdHSP101c* showed that each of Glutamic acid (Glu) , Isoleucine (Ile), Lysine (Lys) were decreased by 25.6%, 35% and 75% respectively. On the other hand Valine (Val), Glycine (Gly), Methionine (Met), Phenylalanine (Phe) and Leucine (Leu) were increased by 14.37%, 17%, 17, 29.8% and 39% respectively.

**C. Analyses of evolutionary divergence:** between sequences *AF174433.1 (HSP101c)*, *AF083344.2\_(HSP101)*, *AF097363.1\_(Hsp101b)*, *Sids12\_HSp101* and *Gemmeiza9\_HSP101* showed that, *Sids12\_HSp101* and *Gemmeiza9\_HSP101* had a great resemblance and similarity with *AF174433.1\_(HSP101c)*. The genetic similarity between *AF174433.1 (HSP101c)* and *Gemmeiza9 HSP101* was 100%. On the other hand the genetic similarity between *AF174433.1 (HSP101c)* and *Sids12\_HSp101* was 99%.

**In general**, Amino acids sequence analysis revealed only effective changes at *Sids\_HSP101* amino acids sequence (1%). Evolutionary, *Sids12\_HSp101* and *Gemmeiza9\_HSP101* had a great similarity with *AF174433.1\_(HSP101c)*.

According to results, it can be concluded that plants in heat stress, make changes in some of their physiological and biochemical features. From this study it can be concluded that tolerant wheat (*Gemmieza9*) Varity exhibited the lower values of relative injury and a slight depression of chlorophyll content and the highest value of proline accumulation under heat stress (35°C), compared to sensitive Varity (*sids12*).

This study has also shown that the physiological and biochemical indices used to evaluate plant response to high temperature were effective in assessing promising bread wheat varieties for heat tolerance. Maintenance of high expression levels of *HSP101* during heat stress in *Gemmieza9* may also be reason for better tolerance of this varity as compared with *Sids12*, as *HSP101* plays a crucial role in repair of heat damaged proteins. No polymorphism was detected in *Gemmeiza9\_HSP101* sequence accompanied by significant increase in proline content and less decrease in chlorophyll content and relative membrane injury.

**In conclusion**, the remarkable response shown by the *Gemmeiza9* varity makes it a good candidate for future breeding to ensure high yield obtained under heat stress conditions. However, there is still need to manipulate other genes controlling HSP production which may be beneficial for breeding heat-tolerant wheat genotypes.