

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Physiological studies

4.1.1 Responses for membrane thermostability in Wheat Varieties

High temperature damages cellular membranes due to lipid peroxidation (**Mandhania et al., 2006**). The integrity and functions of biological membranes are sensitive to high temperature, as heat stress alters the tertiary and quaternary structures of membrane proteins. (**Howarth, 2005**). **Evan (2013)** indicated that, Direct alterations due to high temperatures include protein denaturation and aggregation, therefore protein content declined because heat stress. Decline of protein content may be due to the inhibition of protein synthesis or protein degradation.

In the present study membrane injury was measured for high temperature tolerance through MTS test in six Egyptian wheat varieties is shown in (Fig. 2). Results showed that the highest membrane injury was observed in cultivar Misr1 which was closely followed by varieties Sids12 and Misr2, (82.85, 77.91, 75.246) respectively. **Efeoglu and Terzioglu (2007)** reported that high temperatures at seedling growth decreased MTS in wheat.

These results were in agreement with (**Behl, 1993**) that showed heat damage to plasma membrane destroys membrane integrity causing solute leakage from the cells. The extent of damage may be estimated by conductometric measurement of electrolyte leakage of solute from leaf tissue after a heat shock. The EC is proportional to cell damage. Thus, a heat tolerant genotype shows less EC after heat shock. Also, (**Efeoglu and Terzioglu 2007**) indicated that high temperatures at seedling growth decreased MTS in wheat. On the other hand cultivar Giza168 showed moderate value of membrane injury which revealed (52.57) %.

Sheikh et al. (2010) reported that differences among RI of varieties possibly caused by better maintaining of cellular membrane integrity of some varieties than others under high temperature stress.

The lowest membrane injury value was observed in cultivar Gemmiza 9 and Sakha 93, (45.93, 48.63), respectively, (**Table, 3**). These results were in agreement with **Renuet et al., (2004)** that showed that heat tolerance with respect to cell injury (%) revealed that the genotypes with less injury to plasma membranes are tolerant as compared to the genotypes with more injury to cell membrane. MISR 1, Sids 12, Misr 2 and Giza 168 were considered as heat sensitive (HS) cultivar. The other two wheat cultivar Sakha 93 and Gemmiza9 showed less than 50% MI test and were grouped as heat tolerant (HT) cultivar.

These results were in accordance with (**Ahmed and Hasan 2011**) who stated that wheat cultivar which showed equal or greater than 50% membrane injury were considered as heat sensitive (HS) genotypes and cultivars showed less than 50% MI in membrane thermostability test were grouped as heat tolerant (HT) genotypes.

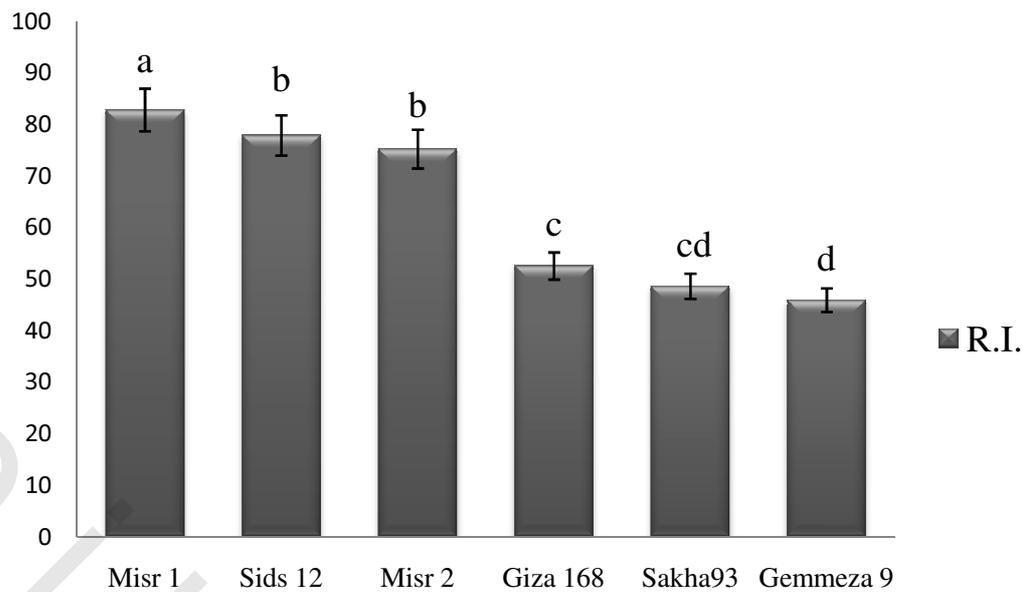


Figure (2): Membrane injury to high temperature of ten days old seedling of six wheat cultivars measured. Values followed by the different letter(s) are significantly different from each other by L.S.D. at 0.05.

Table (3): Effect of heat stress treatment on Seedling Relative injury of six wheat cultivars, Mean (\pm SE).

varieties [A]	Relative injury (%)		
	At 25 °C	At 35 °C	Calibrated ^y
Misr1	14.69 \pm 0.434	86.12 \pm 0.956	82.85 ^a
Sids12	16.09 \pm 0.390	72.88 \pm 1.310	77.91 ^b
Misr2	15.64 \pm 0.228	74.71 \pm 0.921	75.246 ^b
Giza168	17.82 \pm 0.422	32.96 \pm 0.826	52.57 ^c
Sakha93	15.65 \pm 0.911	33.03 \pm 0.642	48.63 ^{cd}
Gemmeiza9	18.45 \pm 0.217	35.93 \pm 0.428	45.93 ^d
Total mean	16.39 ^b	55.938 ^a	63.85
L.S.D. _{0.05}	4.49		

Values, marked with the same alphabetical letters, within comparable of means, do not differ significantly, using L.S.D. at 0.05 level of probability.

*** = Highly significant at 0.05 level of probability.

4.1.2 Chlorophyll content in wheat varieties

High temperature treatment affected the chlorophyll amount of ten days seedlings leaves. Under control conditions no significant changes in chlorophyll content were observed. There was significant reduction in chlorophyll content at all cultivars under heat stress conditions and maximum reduction was recorded in HS varieties. Our results in the same line with some previous studies have shown that heat stress reduces chlorophyll content in wheat, suggesting that temperature stress has a broadly similar effect on Chlorophyll biosynthetic enzymes in seedlings wheat (**Tewari and Tripathy, 1998**). Number of workers (**Almeselmani et al., 2006, Al katib and Paulsen, 1990 and Amani et al., 1996**) has also reported maximum reductions in chlorophyll content due to high temperature exposure.

The reduction of total chlorophyll contents of leaves was detected with enhanced temperature. Total Chlorophyll accumulation was significantly inhibited by 35°C temperature (Fig. 3). The 25°C temperature did not cause any significant changes on chlorophyll amounts. These results were in agreement with Also, **Moaed et al., (2012)** mentioned that heat stress significantly reduced wheat leaf chlorophyll content at all stages of growth. Results in Table (4) showed that 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 cultivar which was closely followed by cultivar sakha93 showed (41 and 53.66) % respectively.

Reynolds et al. (2007) showed premature loss of chlorophyll due to heat sensitivity in wheat crop. Difference in leaf chlorophyll content between tolerant and susceptible wheat genotypes when subjected to high temperature stress has been reported (**Bhanu, 1997**). **Tardy et al. (1998)** reported that the light green color leaves were due to a non-specific decrease in both chlorophyll and Carotenoids, without repartitioning of the pigments between or within the pigmented thylakoid complexes. This pigment alteration appears to be an adaptation to high temperature rather than to strong light.

Current research was also measured to study the effect of heat stress on chlorophyll *a/b* ratio. The reduction in chlorophyll *b* was more compared to chlorophyll *a* under heat stress and thus the chlorophyll *a/b* ratio increased under heat stress in all six wheat cultivars (Fig.4). However the mean of chlorophyll *a/b* ratio was higher at Gemmeiza9 cultivar (2.387) than other wheat varieties, under heat stress the chlorophyll *a/b* ratio was higher in Giza168 (4.971) than other wheat varieties, indicating the hypersensitivity of chlorophyll *b* formation /breakdown in Giza168 than other varieties (Table 5).

This agreement with **Camejo et al., (2005) ; Wahid and Ghazanfar. (2006)** they reported that, the increased of chlorophyll *a/b* ratio and a decreased of chlorophyll: carotenoids ratio were observed in the tolerant genotypes under high temperatures, indicating that these changes were related to thermotolerance. But **Mohammadreza (2012)** who reported that Chlorophylls and carotenoids contents decreased significantly at high temperature stress and reduction of chlorophyll *a* and *b* contents of leaves was detected after 35/30°C day/night temperature treatment in leaves and the amount of chlorophyll *a*, chlorophyll *b* and carotenoids did not show any significant changes at 30/25°C, but a results showed that the ratio of chlorophyll *a/b* and the ratio of chlorophyll/carotenoids has not been changed under heat stress.

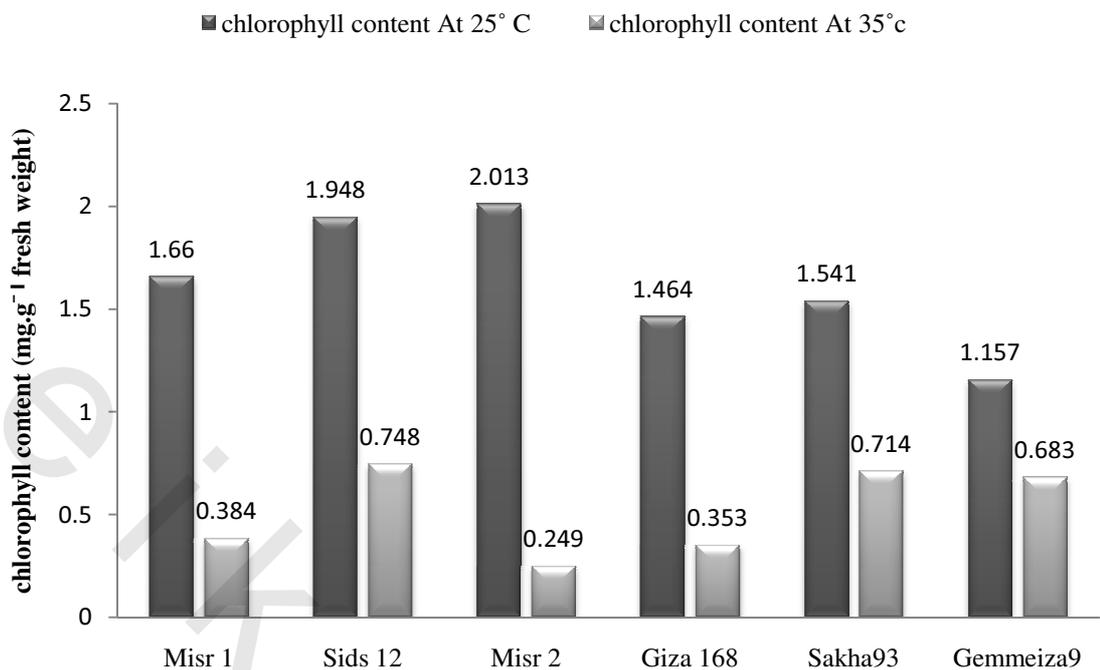


Figure (3): Average seedling chlorophyll content (mg.g⁻¹ fresh weight) at two temperature regimes for six Egyptian wheat varieties.

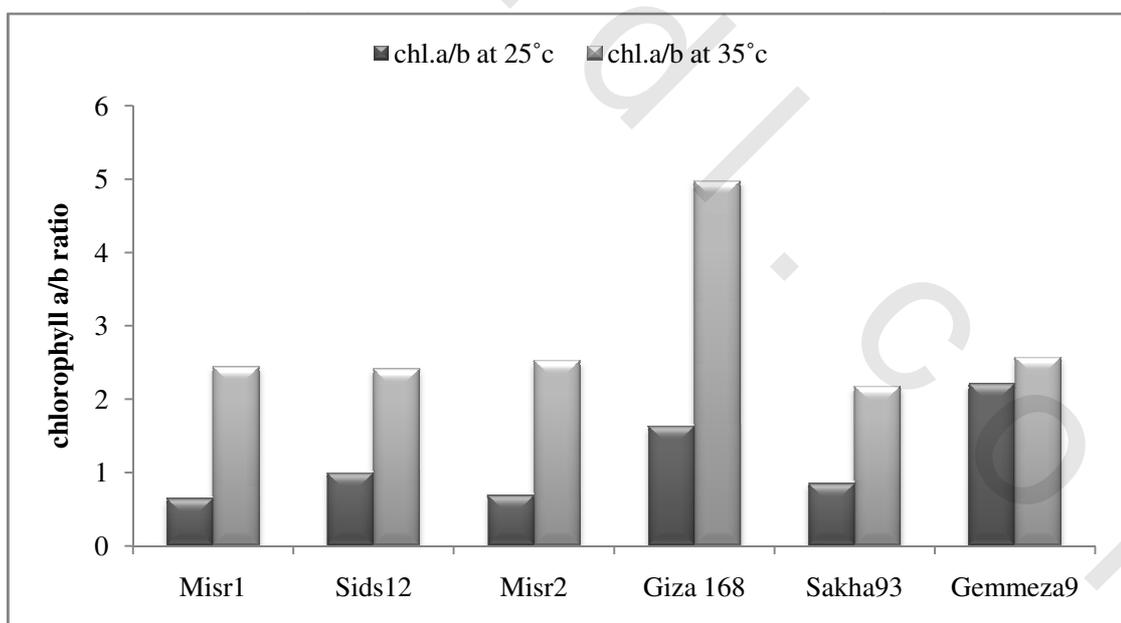


Figure (4): Average seedling chlorophyll a/b ratio under the effect of two temperature regimes for six Egyptian wheat varieties.

Table (4) Effect of heat stress treatment on Seedling total chlorophyll content of six wheat varieties (Mean ± SE)

varieties [A]	chlorophyll content (mg.g fresh weight)				[A]	Significance		
	At 25° C	At 35° C	(%) Chl. Relative to 25°C	(%) Chl. decrease Relative at 35°C	Mean	[A]	[B]	[AxB]
Misr1	1.660 ± 0.004	0.384 ± 0.001	23.1	76.8	1.022 ^d			
Sids12	1.948 ± 0.017	0.748 ± 0.008	38.4	61.6	1.346 ^a			
Misr2	2.013 ± 0.006	0.249 ± 0.003	12.8	87.6	1.131 ^b			
Giza 168	1.464 ± 0.018	0.353 ± 0.006	24.1	75.9	0.909 ^f	**	**	**
Sakha93	1.541 ± 0.005	0.714 ± 0.003	46.3	53.66	1.128 ^c			
Gemmeiza9	1.157 ± 0.001	0.683 ± 0.002	59	41	0.921 ^c			
Total mean	1.63 ^a	0.522 ^b						
L.S.D(0.05)						0.021	0.012	0.105

Values marked with the same alphabetical letters, within comparable of means, do not differ significantly, using L.S.D. at 0.05 level of probability / *** = Highly significant at 0.05 level of probability.

Table (5) Effect of heat stress treatment on Seedling chlorophyll *a/b* ratio of six wheat varieties

varieties [A]	chlorophyll <i>a/b</i> ratio		A	Significance		
	At 25° C	At 35° C	Mean	[A]	[B]	[AxB]
Misr1	0.640 ± 0.05	2.439 ± 0.01	1.539 ^e			
Sids12	0.990 ± 0.04	2.409 ± 0.03	1.699 ^c			
Misr2	0.685 ± 0.01	2.525 ± 0.06	1.605 ^d			
Giza 168	1.628 ± 0.03	4.971 ± 0.02	3.3 ^a	**	**	**
Sakha93	0.847 ± 0.02	2.172 ± 0.01	1.509 ^f			
Gemmeiza9	2.207 ± 0.05	2.567 ± 0.03	2.387 ^b			
Total mean	1.166 ^b	2.847 ^a				
L.S.D(0.05)				0.007	0.004	0.014

Values marked with the same alphabetical letters, within comparable of means, do not differ significantly, using L.S.D. at 0.05 level of probability / *** = Highly significant at 0.05 level of probability.

4.2 Biochemical studies:

4.2.1. Responses for Proline content in Wheat cultivars

Plants usually accumulate some compatible solutes with low molecular mass such as proline (**Ashraf and Harris, 2004**). It has been shown that accumulation of proline is a common response to a wide range of biotic and abiotic stresses such as salt (**Aghaei *et al.*, 2009**), drought and high temperature (**Kumar *et al.*, 2012**).

The results of present research showed that increasing temperature lead to increase of proline content of ten days seedlings and were influenced significantly by the interaction effect of temperature regimes and Egyptian wheat cultivars.

Results showed that seedling proline content at 35°C was higher compared to those at 25° C (**Fig. 5**). The increments of seedling proline content from 25 to 35° C were significant for all wheat cultivar (**Table 6**). These results confirm the previously observation of **Kumar (2012)**. This results also agreement with (**Ronde *et al.*, 2001**) showed that proline accumulation in high temperature has been reported in cotton leaves. Under supra-optimal temperature genotypic difference in proline accumulation pattern has also been reported in six cotton cultivars and in apple (**Park *et al.*, 2001**) and in flag leaves of wheat (**Hasan *et al.*, 2007**).The highest mean seedlings proline content was observed cultivar Gemmeiza9 which was closely followed by varity Giza 168, (1.824, 1.747) respectively.

Results indicated that, the lowest mean value was observed in Misr 2 (0.833). The increment of proline level from 25 to 35° C is indicated by the relative value. At 35° C Giza 168, Sakha 93 and Gemmeiza 9 varities produced more than double (> 200%) proline than that at 25° C. The highest relative value was observed in cultivar sakha 93, (246).

On the other hand, Misr 1, Sids 12 and Misr 2 varities produced less than double (< 200%) proline at 35° C compared to that at 25°C (**Fig. 6**).This result was supported by **Ahmed and Hasan (2011)** they mentioned that the increment of proline level from 25 to 35° C in different wheat genotypes is indicated by the relative value. At 35° C the HT genotypes produced more than double (> 200%) proline than that at 25°C. On the other hand, the HS produced less than double (< 200%) proline at 35° C compared to that at 25°C.

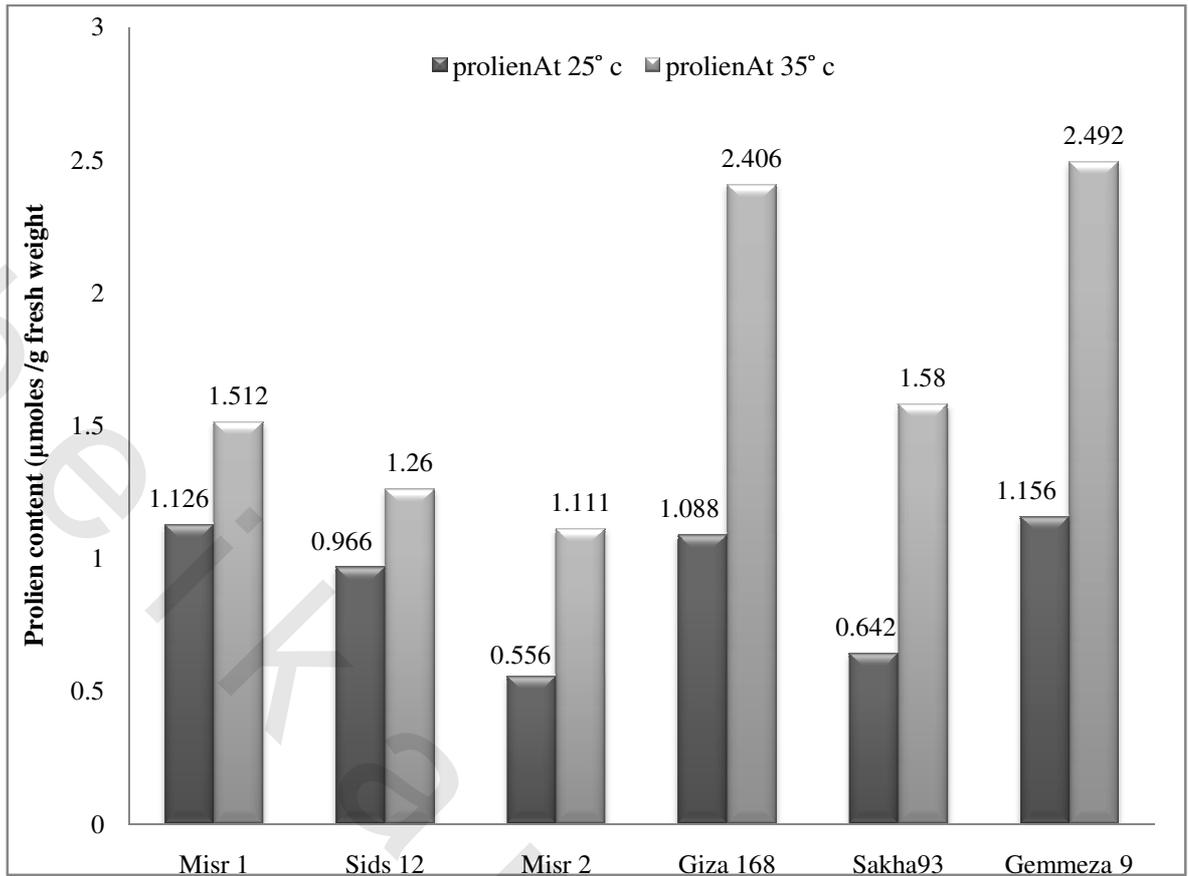


Figure (5): Average seedling proline content (µmoles/g fresh weight) at two temperature regimes for six Egyptian wheat varieties.

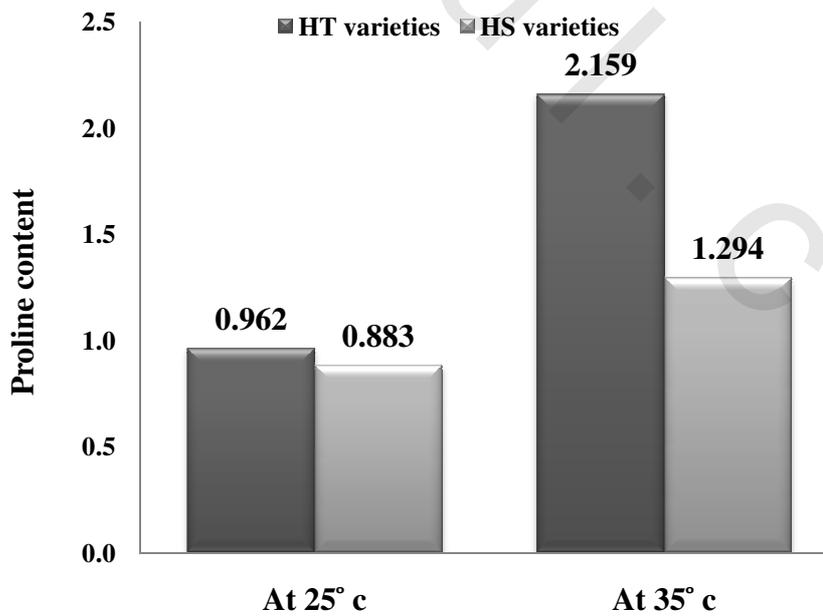


Figure (6): Average seedling proline content (µmoles/g fresh weight) of heat tolerant and heat sensitive wheat varieties as influenced by temperature regimes.

Table (6) Effect of heat stress treatment on Seedling Proline content ($\mu\text{moles /g fresh weight}$) for six wheat varieties (Mean \pm SE)

Varieties [A]	Seedling Proline content ($\mu\text{moles /g fresh weight}$)				Significance		
	At 25° C	At 35° C	Relative to 25°C (%)	Mean	[A]	[B]	[AxB]
Misr 1	1.126 ^g \pm 0.058	1.512 ^d \pm 0.036	134	1.319c			
Sids 12	0.966 ⁱ \pm 0.012	1.26 ^e \pm 0.014	130	1.111d			
Misr 2	0.556 ^l \pm 0.019	1.111 ^h \pm 0.061	199	0.833e			
Giza 168	1.088 ⁱ \pm 0.053	2.406 ^c \pm 0.009	221	1.747b	**	**	**
Sakha93	0.642 ^k \pm 0.018	1.58 ^c \pm 0.065	246	1.113d			
Gemmeiza9	1.156 ^f \pm 0.024	2.492 ^a \pm 0.037	216	1.824a			
Total mean	0.95 ^b	1.72 ^a					
L.S.D._{0.05}					0.051	0.029	0.102

Values, marked with the same alphabetical letters, within comparable of means, do not differ significantly, using L.S.D. at 0.05 level of probability.

*** = Highly significant at 0.05 level of probability.

4.3 Correlation and regression analysis

The study of the percentage of relative content of membrane injury, proline content and chlorophyll content (**Table 2, 3 and 4**) revealed that wheat heat sensitive seedlings had 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) respectively (**Figure 7, 8 and 9**). These results were in agreement with **Ahmed and Hasan (2011)** they found insignificant ($r = -0.155$) between proline content in 20 wheat genotypes at 25° C and % membrane injury.

On the other hand, (relative membrane injury & relative seedling proline content), (relative membrane injury & relative chlorophyll content) and (relative seedling proline content & relative chlorophyll content) maintained a strong positive correlation ($r = 0.728$), ($r=0.51$) and strong negative correlation ($r = -0.966$) respectively across the heat tolerant wheat varieties (HT) (**Figure 10, 11 and 12**). These results were agreement with **Ahmed and Hasan (2011)** they indicated that, the increased seedling proline level due to high temperature can be used to screen HT wheat genotypes, which is comparable to cell membrane thermostability test. The correlation between (relative seedling proline content & relative chlorophyll content) in heat sensitive (HS) wheat seedling was strong and in heat tolerant wheat varieties (HT) was very strong. These results indicated by **KHAN (2009)** who observed that the salinity tolerant genotypes have higher proline accumulation, high K/Na ratio and less chlorophyll degradation as compared to sensitive ones. Also, **Keyvan (2010)** showed a significant correlation ($r= -0.695$), ($r= -0.826$) and ($r= -0.728$) between proline and (Chlorophyll a, chlorophyll b and total chlorophyll) respectively across the wheat varieties under drought stress.

In general, we noticed a strong correlation between the increase of proline content and chlorophyll content in relation to relative membrane injury especially under the stress of heat. The change from 25°C to 35°C resulted in an increase of membrane injury by 62.3 % in wheat heat sensitive varieties compared to heat tolerant varieties. In contrast, proline content and chlorophyll content were increased by 67.8% and 57% respectively in wheat heat tolerant varieties compared to heat sensitive varieties (Table 7).

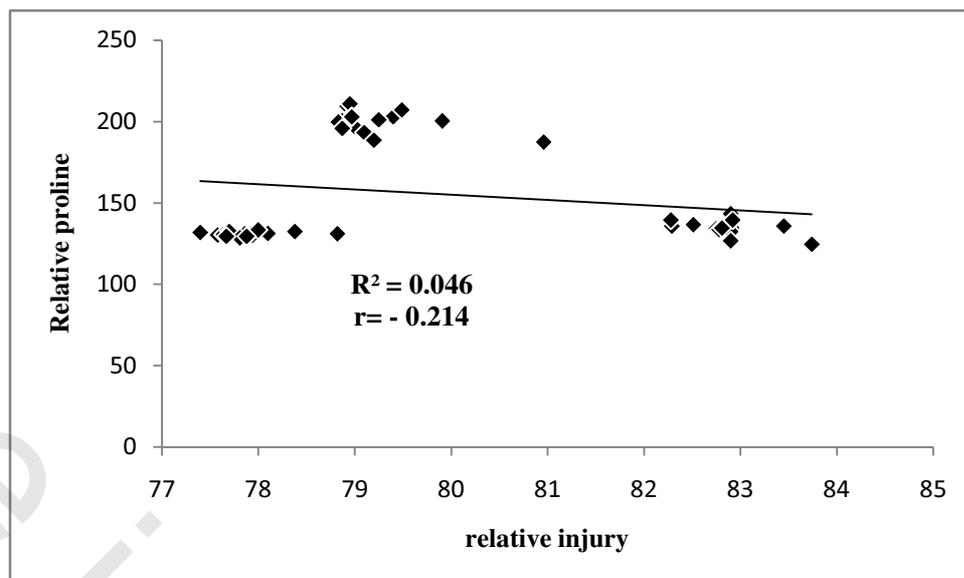


Figure (7): Relationship ($r = -0.214$) between relative membrane injury and relative seedling proline content in heat sensitive wheat varieties.

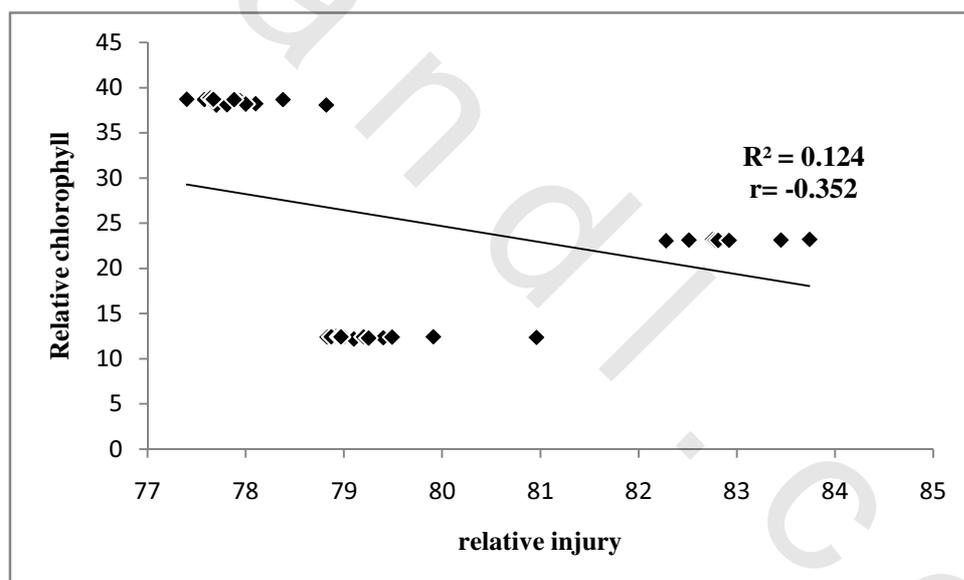


Figure (8): Relationship ($r = -0.352$) between relative membrane injury and relative chlorophyll content in heat sensitive wheat varieties.

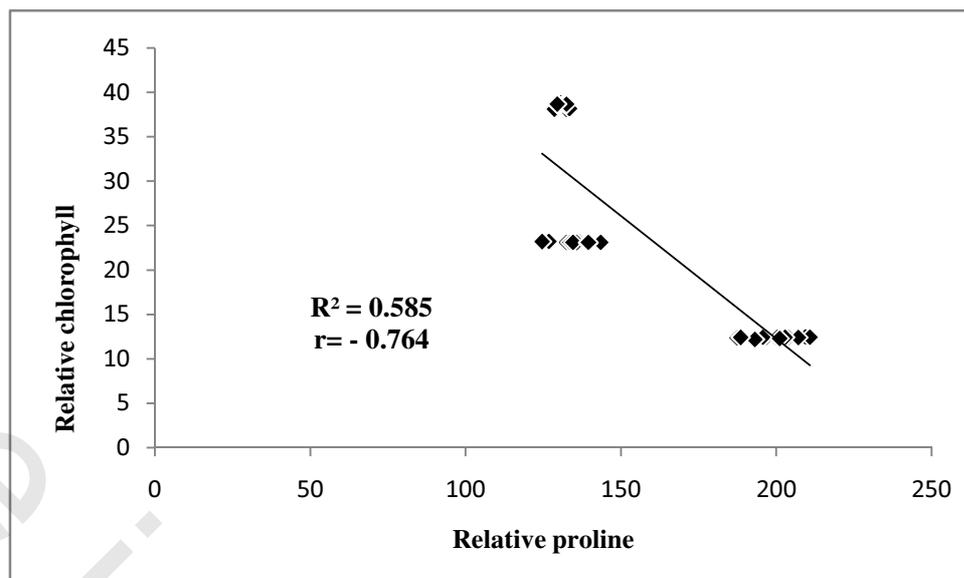


Figure (9): Relationship ($r = -0.764$) between relative seedling proline content and relative chlorophyll content in heat sensitive wheat varieties.



Figure (10): Relationship ($r = 0.728$) between relative membrane injury and relative seedling proline content in heat tolerant wheat varieties.

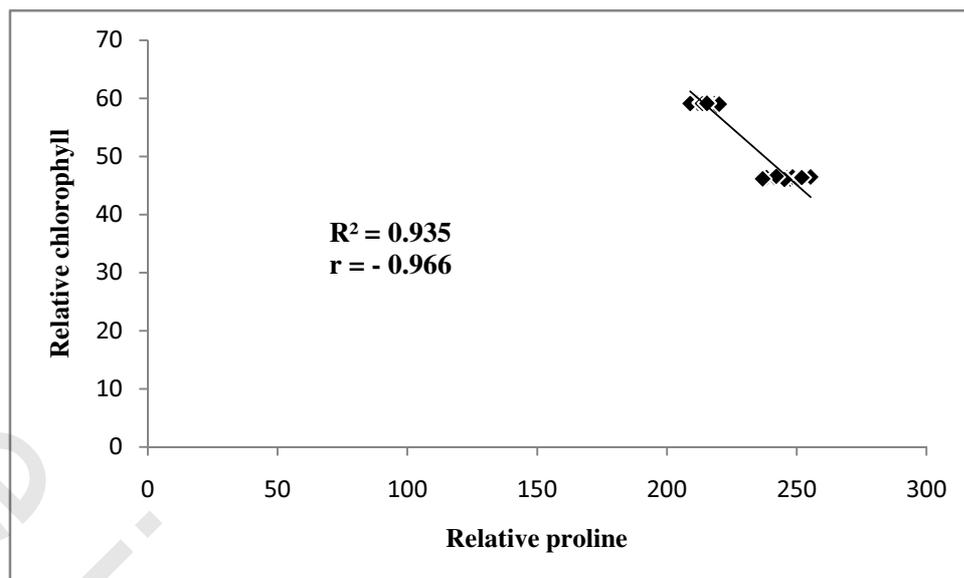


Figure (11): Relationship ($r = -0.966$) between relative seedling proline content and relative chlorophyll content in heat tolerant wheat varieties.

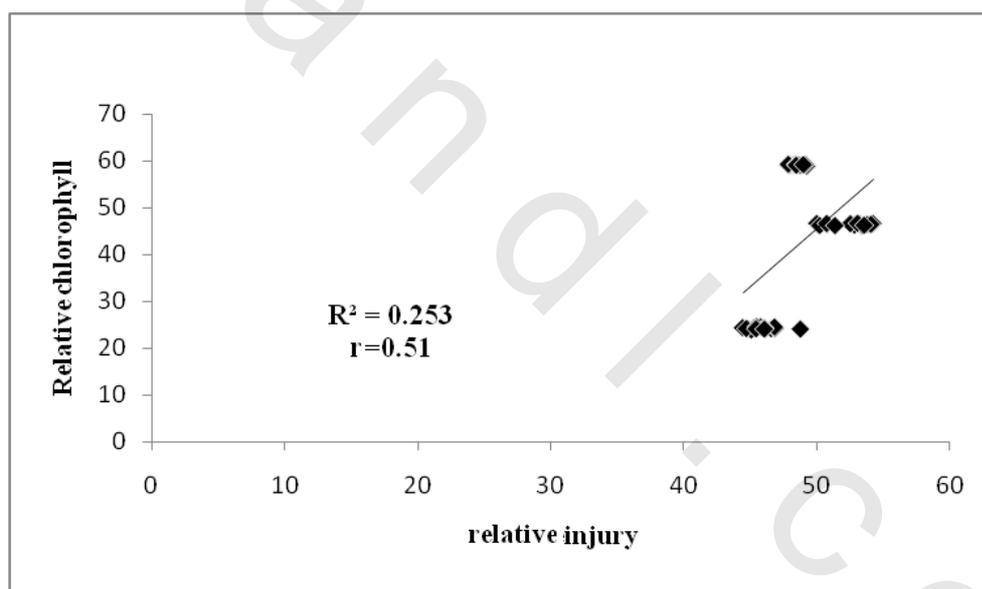


Figure (12): Relationship ($r = 0.51$) between relative membrane injury and relative chlorophyll content in heat tolerant wheat varieties.

Table(7): Relative change % between Heat Sensitive varieties to Heat tolerant varieties through Relative injury, relative proline content and relative chlorophyll content

Measurement	Heat Sensitive varieties			Mean	Heat tolerant varieties			Mean	Relative change %
	Misr1	Sids12	Misr 2		Giza 168	Sakha93	Gemmeiza 9		
Relative injury	82.85	77.91	75.24	78.6	52.57	48.63	45.93	49	62.3
Relative Proline content	134	130	199	154.3	221	246	216	227.6	67.8
Relative Chlorophyll content	23.1	38.4	12.8	24.7	24.1	46.3	59	43.1	57

4.4. Molecular Genetics studies

4.4.1 HSP101 gene transcript measurement by Quantitative Real-time PCR amplification

In order to investigate whether the *HSP101* gene is different not only in structure but also in function, in the present work we have studied the expression profiles in different conditions, with the aid of quantitative transcriptomic tools like Quantitative (Real-Time) RT-PCR. Quantitative RT-PCR revealed that *HSP101* is expressed constitutively in all wheat varieties control and treatment by heat until 14 days after germination (**Figure 13**). Consistent with these observations, in wheat, HSP101 protein was detected in leaves until 7 days after germination, while in maize it was detected until 12 days after germination under normal growth temperatures (**Young et al. 2001**).

Campbell et al. (2001) shown that HSP101 in wheat is induced by heat and drought. Expression of *HSP101* is heat-inducible in leaves of rice (**Agarwal et al. 2003**) and maize (**Nieto-Sotelo et al., 2006**). In leaves of lima bean, the HSP100/ClpB protein gene constitutively expressed, but transcript levels increased under heat stress (**Keeler et al. 2000**). Genetic and transgenic analyses have shown that HSP101 is essential for acquired as well as basal thermotolerance (**Queitsch et al. 2000; Hong and Vierling 2001; Nieto-Sotelo et al. 2006; Agarwal et al. 2003; Katiyar- Agarwal et al. 2003**). It has been reported that HSPs play a general role is to act as molecular chaperones regulating the folding and accumulation of proteins as well as localization and degradation in all plants and animal species (**Panaretou and Zhai 2008; Hu et al. 2009; Gupta et al. 2010**).

In heat tolerant variety 'Gemmeiza 9' expression level of *HSP101* showed at general high expression at two heat treatments (S and A24+S). In contrast, heat susceptible genotype 'Sids12' showed considerable reduction as general in *HSP101* transcripts at heat treatments (S and A24+S) of heat stress as compared with their other varieties (**Table 8**) heat-tolerant variety sakha93 which was closely followed by varieties Misr1 and Gemmeiza9, (192.7, 143.1, 126.2) % respectively showed up regulation of *HSP101* expression under heat stress treated at 42°C for 2 h (S). In contrast, variety Giza168 did not show *HSP101* high transcripts under this heat stress treatment (**Figure 14; A**). Also, in this study, heat-tolerant variety Gemmeiza9 which was followed by variety Misr1 high expressed (429.7, 220.3) % of *HSP101* expression under heat stress treated at 34°C for 24 h + 2h at 42°C (A24+S). But, variety sids12 show the least *HSP101* transcripts (6.6) % under this heat stress treatment (**Figure 14; B**). It has been reported that there is genetic variability in the synthesis of HSPs in wheat (**Ahn 2004 and Efeoglu 2009**). HSP high transcript of *HSP101* was maintained at all heat stress treatments only in heat tolerant varieties 'Gemmeiza9 and Misr1' but not in heat sensitive cultivar 'Sids12'. Since HSP101 is essential for thermotolerance, heat tolerance of varieties 'Gemmeiza9 and Misr1' are at least in part due to *HSP101* expression heat stress.

Tripp et al. (2009) suggested that these proteins as chaperones prevent the irreversible aggregation of other proteins and participate in refolding proteins during heat stress conditions. HSP104/HSP101 mediates the resolubilization of heat-inactivated proteins from insoluble aggregates (**Parsell et al. 1994; Agarwal et al. 2003**). Moreover, these results showed seedling exposed to adaptation (34°C for 24 h+2h at 42°C) induced over expression of HSP101 with total average (181.5%) by about (59.6%) increment than seedling treated 2h at 42°C directly with total average (113.7%) , this means that over

expression of HSP101 requires adaptation of wheat seedlings before treatment (**Fig.15**). **Moaed *et al.*, (2012)** showed that maintenance of high expression levels of *HSP101* during long-term heat stress in 'C306' may also be reason for better tolerance of 'C306' as compared with 'PBW343', as HSP101 plays a crucial role in repair of heat damaged proteins.

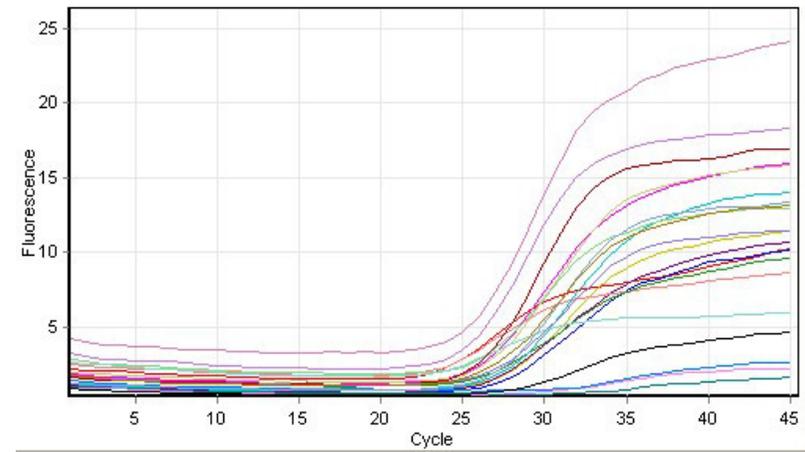
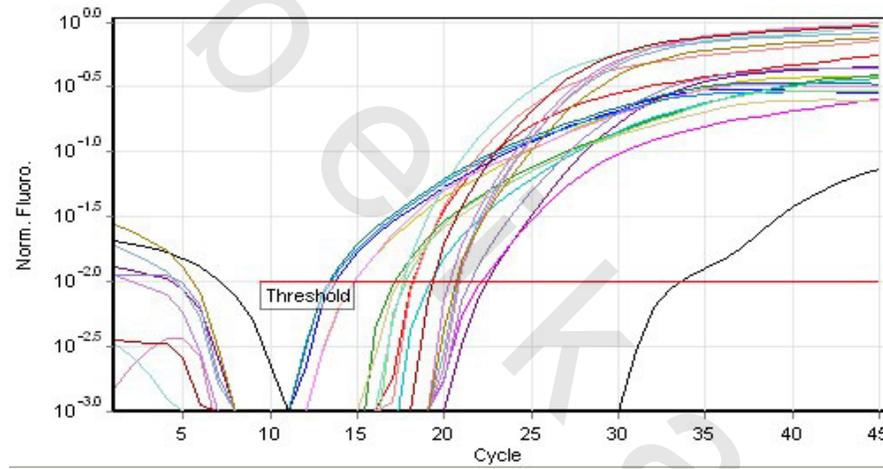
Table (8): Analysis the means of Relative HSP101 Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method (Kenneth J. Livak and Thomas D. Schmittgen., 2001).

varity	Treatment	Average Ct HSP101 Gene Expression	Average Ct reference Gene expression	Average Ct control expression	$\Delta\Delta CT$	$2^{-\Delta\Delta C_T}$ Ratio target gene expression	%
Misr1	S	20.53	17.55	3.5	-0.51	1.431	143.1
	A24+S	19.91		3.5	-1.14	2.203	220.3
Sids 12	S	21.13	18.01	21.19	-0.062	1.044	104.4
	A24+S	25.1		21.19	3.90	0.066	6.6
Misr2	S	14.64	18.12	14.30	0.34	0.79	79
	A24+S	13.47		14.30	-0.83	1.777	177.7
Giza178	S	20.85	18.44	19.41	1.44	0.368	36.8
	A24+S	19.31		19.41	-0.10	1.072	107.2
Sakha93	S	19.263	18.18	20.21	-0.94	1.927	192.7
	A24+S	19.65		20.21	-0.56	1.474	147.4
Gemmeiza9	S	19.33	18.18	19.66	-0.34	1.262	126.2
	A24+S	17.56		19.66	-2.10	4.297	429.7

Seedlings treated at 42°C for 2 h (S) and s treated at 34°C for 24 h + 2h at 42°C (A24+S).

(S) Treatment mean =113.7 (A24+S) Treatment mean =181.5

(A)



(B)

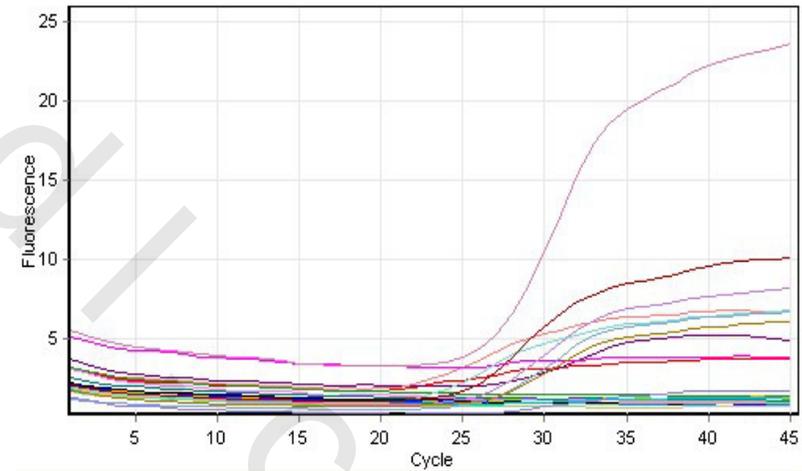
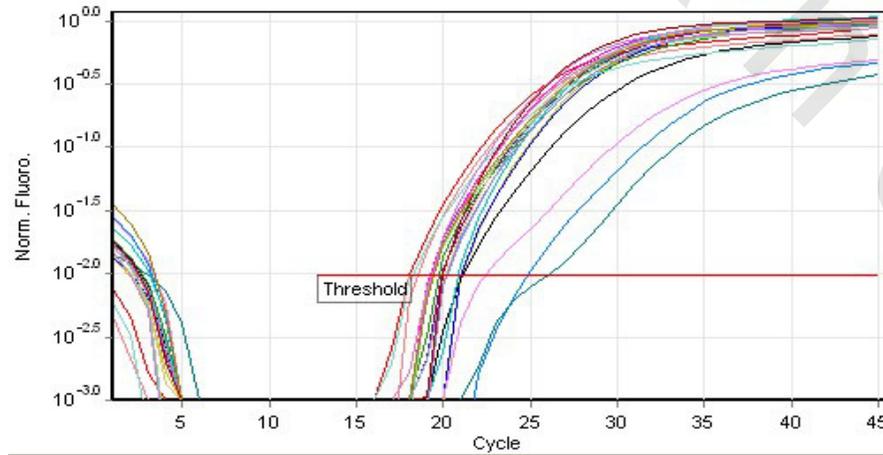
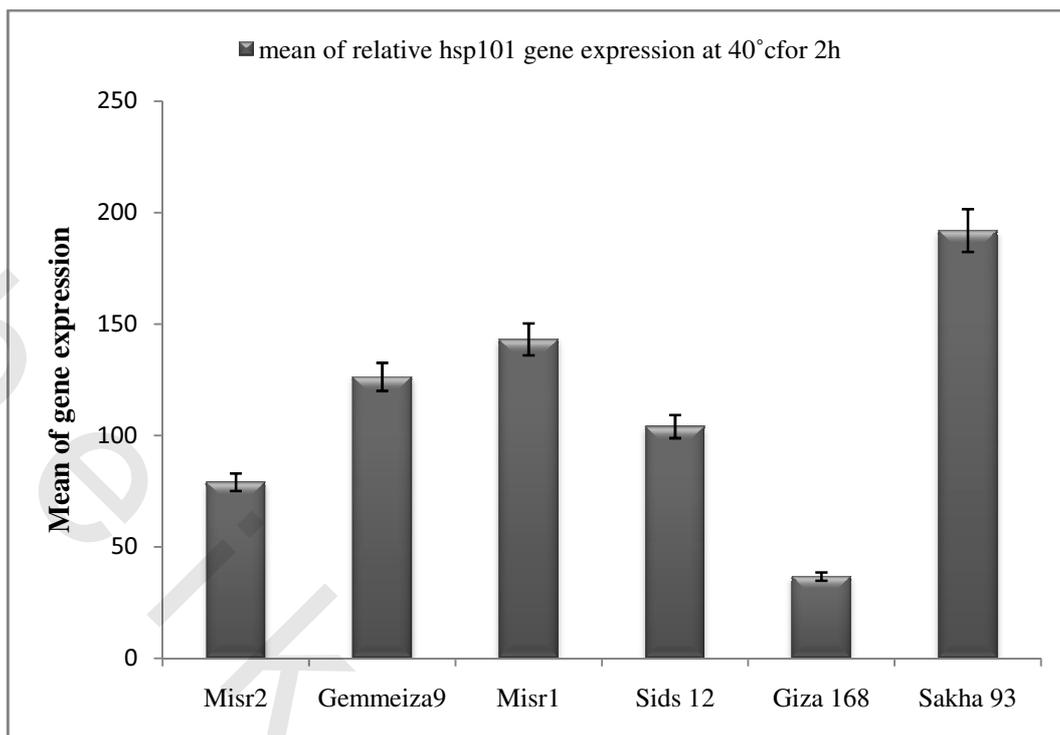
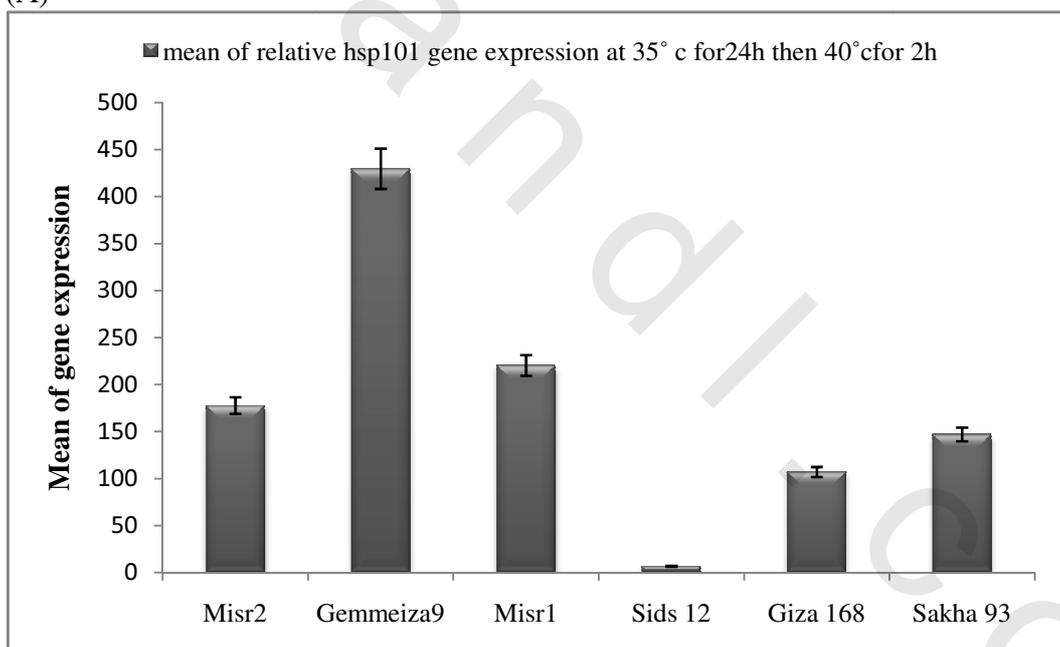


Figure (13): Quantification data by using HSP101 F3+R3 primers for HSP101 gene expression with β -Actin reference gene for RT-PCR cycling with SYBER-Green (A+B).



(A)



(B)

Figure (14): Quantification of gene expression of HSP101 gene at six wheat varieties.

A. Mean of relative gene expression of HSp101 at six varieties treated at 40°C for 2h.

B. Mean of relative gene expression of HSp101 at six varieties treated at 34°C for 24 h + 2h at 42°C.

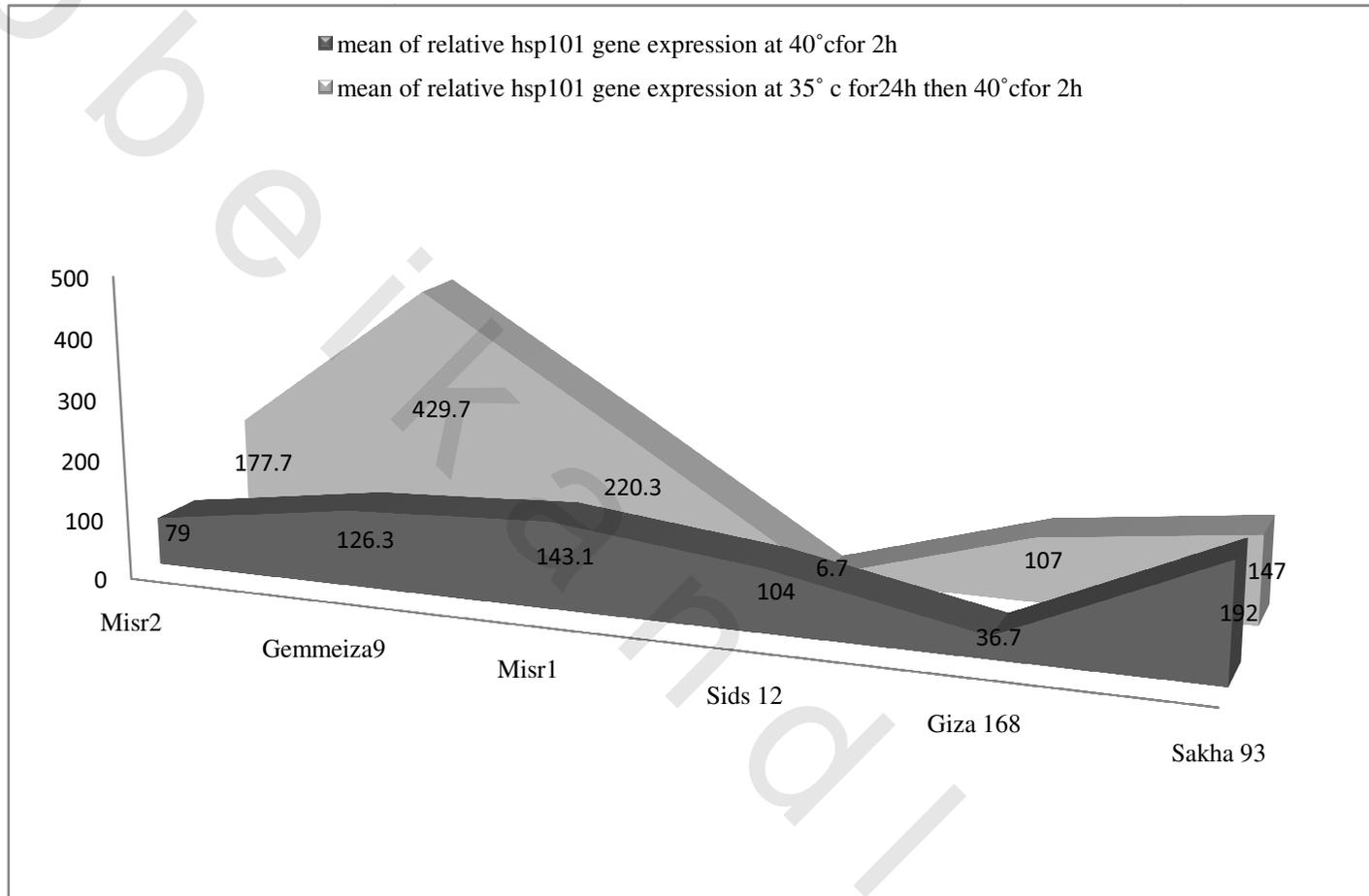


Figure (15): Relationship between HSp101 gene expression level and heat treatment.

4.4.2. Bioinformatics Studies

4.4.2.1. SNP identification and frequencies

Single nucleotide polymorphisms are single base pair positions in genomic DNA at which different sequence alternatives (alleles) exist in normal individuals in some population(s), wherein the least frequent allele has an abundance of at least 1% or greater. Thus, single base insertion/deletion variants (InDels) would not be considered to be SNPs (Brookes, 1999).

Two sequences aligned (*HSP101_Sids2* and *HSP 101_Gemmeiza9*) with Anchor sequence HSP101c by using the DNAMAN® software (Lyon BioSoft, Quebec, Canada) (Fig. 18 A, B and C) showed that multiple SNPs existed manually by calculate the different loci which shading in the 3 nucleotide sequences. The comparison of these sequences allowed the detection of SNPs (Feltus *et al.* 2004).

In *sids12 hsp101* sequence 11 transition from `A` to `G` and 5 transversion loci represented 4 loci from `A` to `T` and one loci from `C` to `G` were noticed. Two deletions (A nucleotides) were noticed and represented (11%) of the total mutations of total length 1795 bp.

No polymorphism was detected in *Gemmeiza9 HSP101* sequence with the exception of two `G` nucleotide deletions of the total mutations, (Table 9). In *sids12*, Transition from `A` to `G` was the most frequent event, accounting for 61% of the total mutations. On the other hand, transversion was accounting less frequency at 28% of the total mutations. The SNP frequency was about one SNP per 112 bp and one InDel per 898 bp (Table 10).

SNPs finder database allowed detection various sequence polymorphisms (SNPs, insertion, deletion polymorphism and short tandem repeats) coming from different sequencing or genotyping technologies and obtained on different species. This release is available at <http://snpsfinder.lanl.gov>. Generating multiple sequence alignments and detecting SNPs automated taking into consideration the quality of the sequences as well as the locations of the predicted SNPs to assist further evaluation of the predicted SNPs (Song, *et al.*, 2005).

Two sequences (*HSP101_Sids12* and *HSP101_Gemmeiza9*) Fasta format file were uploaded at SNPs finder program and compared with Anchor fragment TaHSP101c (GeneBank Accession Nos. AF174433) (Fig.19 A, B and C). All homologous regions among three sequences were identified after the elimination of paralogous sequences from consideration to reduce false positive SNPs identification.

In this study 8 Transtions from (A) to (G), 2 transversions from (A) to (T) and 3 InDels were detected at *HSP101_Sids12* sequence and one InDel at was detected at *HSP101_Gemmeiza9* sequence, (Table 11). The *HSP101_Sids12* SNPs distribution was: (66%) transitions, (17%) transversions and (17%) InDel (Table 12).

This result is confirmed by Marine (2009) who stated that the single substitution is sufficient to increase the number of hydrogen bonds in the warm adapted ortholog, thus leading to enhanced thermal stability in binding properties and structure. The *HSP101_Sids12* SNP frequency was about one SNP per 180 bp and one InDel per 898 bp.

On the other hand, it can be noticed that the results of automated method were almost in agreement with the manual accounting especially for the SNPs distribution. Moreover, transitions from 'A' to 'G' was the most frequent event, accounting more than 60% of the total mutations at total length of 1795 bp. These were in accordance with **Wakeley (1996)** who showed that all DNA sequences from any genome examined, transitions (T > C, A > G) have been noted to occur at higher frequencies than transversions (T > A, T > G, C > A, C > G).

Sequence 1 > HSP101 gene

```
AGGCGCTCGCGGCGGCGCACGAGATGGCGTCCGAGGCCGGCCACGCGCAGCTCACGC
CGCTGCACCTCGCCGCGGCGCTCGCGGCGGACGGGTCCGGGCATCCTCCGCCAGGCCAT
CGCCACGCGTCCGGCGGCAACGACGCCGCGGCCGAGTCGTTTCGAGCGCGTCGCGTCC
GCCGCGCTCAAGCGGCTGCCCTCGCAGTCCCCGCCGCCCGACACCGTCCCGGCCTCCG
CCTCGCTGGTCAAGGCCGTCCGCCGCGCGCGGTCCGGCGCAGAAGTCGCGCGGGCGACTC
GCACCTCGCCGTCGACCAGCTGCTCATGGGCCTCCTCGAGGACCCGACAGATCTCCGAC
GCGCTCAAGGAGGCCGGCATCTCCGCTGCGCGGGTGAAGGCCGAGGTTCGAGAAGCTC
CGGGGTGGCGACAACCGGCGCGTGGAGTCCGCGTCCGGGGACACCAACTTCCAGGCC
CTCAAGACGTACGGCCGCGACCTCGTGGAGGTGGCGGGCAAGCTGGACCCGGTTCATC
GGCCGCGACGAGGAGATCCGGCGCGTGGTGGGATCCTGTCCGCGGCGCACAAAGAAC
AACCCCGTCTCGTCCGGCGAGCCCGGCGTGGGCAAGACCCCGTGGTGGAGGGGCTC
GCGCGGCGCGTCCGTGCGCGGCGACGTCCCAGCAACCTCCTGGACGTGCGCCTGGTCCG
CGCTCGACATGGGCGCGCTCGTGGCCGGCGCCAAGTACCGCGGCGAGTTCGAGAGCG
GCTCAAGGCCGTGCTCAAGGAGGTGGAGGAGGCCGAGGGGAAGGTGATACTGTTTCAT
CGACGAGATACACCTGGTGTCCGGCGCCGGGCGGACGGAGGGGTCAATGGACGCGGC
CAACCTGTTCAAGCCGATGCTGGCGAGGGGGCGGCTCAGGTGCATTGGCGCGGCGAC
CCTCGAGGAGTACAGGAAGTACG TCGAGA
```

(A)

Sequence 2 > HSP101 gene

```
CATGTGGCGGTGTTCAACACTCTGCTCCAGGTCCTGGACGATGGGCGGTTGAC
CGACGGGCAAGGCAGGACGGTTGATTTTAGGAACACGGTGATCATCATGACCT
CAAACCTTGGCGCGGAGCACCTCCTCGCCGGGATGGTGGGCATTTTCGATGAAG
GTCGCTCGTGATCTGGTCATGCAGGAGGTGAGGAGGCATTTCCGCCCGGAGCT
GCTGAACCGTCTGGACGAGATCGTCATCTTCGACCCTCTGTTCGCATGAGCAGCT
GCGGAGGTTCGCTCGGCTTCAGATGAAAGATGTGGCAGTCCGTCTTGCCGAGAG
GGGCGTTGCTCTGGCCGTCACCGACGCCGCCCTGGACGTCATCCTGTCACTGTC
TTACGATGCGGTCTATGGCGCCGGGCCAATCCGGAGATGGATCGAGAAGAGG
ATAGTGACGGAGCTCTCCAAGATGTTGATCCGCGAGGAGATCGACGAGAACTC
CACGGTGTACATCGACGCTGCGCCCAGCAAGGACGAGCTGACCTATGGCGTCCG
ACAAGCACGGAGGGCTGGTGAACGCGCGCACGGGCCACAAGTCCGACATCCT
GATCCAGGTTCTAGCGGAGCTGTTGGGGGCGATGCGGCGCACGCCGTGAAGA
AGATGTAGATCATGCAGGACAGCGGAGAGGTGGACGACGTGGGGGAAGAGTA
GATGGAAACCGCATCGATTGATCCCTTTCTGACTGTAGTTCCAAGTGCCTGCAC
CAGGCAGTCGTATGAGCTCTGTTTTGCTTTTGGACCGTGCTGATTTAGGTAGT
TCGGAATCGTATCTGTTGAATTGGGGGTGAAATGATTTGTAGTTGAGGATGG
```

(B)

Figure (16): (A and B) partial sequences for the cDNA of HSP101 gene for Sids12 wheat variety.

Sequence 3 > Hsp101 gene

```
GGCGCTCGCGGCGGCGCACGAGATGGCGTCCGAGGCCGGCCACGCGCAGCTC
ACGCCGCTGCACCTCGCCGCGGCGCTCGCGGCGGACAGGTCGGGCATCTCCG
CCAGGCCATCGCCCACGCGTCCGGCGGCAACGACGCCGCGGCCGAGTCGTTCCG
AGCGCGTTCGCGTCCGCCGCGCTCAAGCGGCTGCCCTCGCAGTCCCCGCCGCC
GACACCGTCCCGGCCTCCACCTCGCTGGTCAAGGCCGTCCGCCGCGCGCAGTC
GGCGCAGAAGTCGCGCGGGCGACTCGCACCTCGCCGTCGACCAGCTGCTCATGG
GCCTCCTCGAGGACCCGCAGATCTCCGACGCGCTCAAGGAGGCCGGCATCTCC
GCTGCGCGGGTGAAGGCCGAGGTTCGAGAAGCTCCGGGGAGGCGACAACCGGC
GCGTGGAGTCCGCGTCCGGGGACACCAACTTCCAGGCCCTCAAGACGTACGGC
CGCGACCTCGTGGAGGTGGCGGGCAAGCTGGACCCGGTCATCGGCCGCGACG
AGGAGATCCGGCGCGTGGTGC GGATCCTGTGTCGCGGCGCACAAAGAACAACCC
CGTCCTCATCGGCGAGCCCGGCGTGGGCAAGACCGCCGTGGTGGAGGGGCTCG
CGCAGCGCGTTCGTGCGCGGCGACGTCCCCAGCAACCTCCTGGACGTGCGCCTG
GTCGCGCTCGACATGGGCGCGCTCGTGGCCGGCGCCAAGTACCGCGGCGAGTT
CGAGGAGCGGCTCAAGCCGTGCTCAAGGAGGTGGAGGAGGCCGAGGGGAAGG
TGATACTGTTTCATCGACGAGATAACCTGGTGTCTCGGCGCCGGGCGGACGGAG
GGGTCAATGGACGCGGCCAACCTGTTCAAGCCGATGCTGGCGAGGGGGCAGCT
CAGGTGCATTGGCGCGACGACCCTCGAGGAGTACAGGAAGTACGTCGAGA
```

(A)

Sequence 4 > Hsp101 gene

```
CGCATGTGGCGGTGTTCAACACTCTGCTCCAGGTCCTGGACGATGGGCGGTTG
ACCGACGGGCAAGGCAGGACGGTTGATTTTAGGAACACGGTGATCATCATGAC
CTCAAACCTTGGCGCGGAGCACCTCCTCGCCGGAATGGTGGGCAATTCGATGA
AGGTTGCTCGTGATCTGGTCATGCAGGAGGTGAGGAGGCATTTCCGCCCGGAG
CTGCTGAACCGTCTGGACGAGATCGTCATCTTCGACCCTCTGTGCGATGAGCAG
CTGCGGAAGGTCGCTCGGCTTCAGATGAAAGATGTGGCAGTCCGTCTTGCCGA
GAGGGGCGTTGCTCTGGCCGTCACCGACGCCGCCCTGGACGTCATCCTGTCAC
TGTCTTACGATCCGGTCTATGGCGCCAGGCCAATCCGGAGATGGATCGAGAAG
AGGATAGTGACGGAGCTCTCCAAGATGTTGATCCGCGAGGAGATCGACGAGA
ACTCCACGGTGTACATCGACGCTGCGCCCAGCAAGGACGAGCTGACCTATGGC
GTCGACAAGCACGGAGGGCTGGTGAACGCGCGCACGGGCCACAAGTCCGACA
TCTTGATCCAGGTTCCCTAGCGGAGCTGTTGGGGGCGATGCGGGCGCACGCCGTG
AAGAAGATGAAGATCATGCAGGACAGCGGAGAGGTGGACGACATGGAGGAA
GAGTAGATGGAAACCGCATCGATTATCCCTTTCTGACTGTAGTTCCAAGTGCCT
GCACCAGGCAGTCGTATGAGCTCTGTTTTTGGTGGTGGACCGTGCTGATTTAGG
TAGTTCGGAATCGTATCTGTTGAATTGGGGGTGAAATGAATTGTAGTTGAGGA
TGG
```

(B)

Figure (17): (A and B) partial sequences for the cDNA of HSP101 gene for Gemmeiza9 wheat variety.

Table (9): SNPs analysis manually: Two sequences (Sids2 HSP101 and Gemmieza9 HSP 101) characterization by Alignment with AF174433.1 *TaHSP101c* gene by using DNAMAN® software

Gene Coordinates	SNP Bases by varity			Insertion/deletion (InDel)		
	AF174433.1 <i>TaHSP101c</i>	Gemmiza HSP101	Sids HSP101	AF174433.1 <i>TaHSP101c</i>	Gemmiza HSP101	Sids12 HSP101
217	A	A	G			
358	A	A	G			
389	A	A	G			
537	A	A	T			
715	A	A	G			
764	A	A	G			
870				A	A	-
882				G	-	G
1020	A	A	G			
1040	A	A	G			
2283	A	A	G			
2294	A	A	T			
2416				A	A	-
2528	C	C	G			
2542	A	A	G			
2788	A	A	T			
2821	A	A	G			
2825	A	A	G			
2854				G	-	G
2978	A	A	T			

Table (10): Summary of single nucleotide polymorphism (SNP) characterization manually

SNPs	Gemmiza9 HSP101	%	Sids 12 HSP101	%
Transition	-	0%	11	61%
Tranversion	-	0%	5	28%
(InDel)	2	100%	2	11%
Frequency (bp/SNP)	-		112	
Frequency (bp/InDel)	898		898	

Table (11): SNPs Analysis automated: Anchor AF174433.1 (*Triticum aestivum* heat shock protein 101 (HSP101c) mRNA complete cds) and 2near neighbors sids12 HSP101 and Gemmeiza9 HSP101 by using SNPs Finder program <http://snpsfinder.lanl.gov> (Song, J *et al.*, 2005)

Anchor Sequence Fragment	Gene Coordinates		SNPs	InDels	Gene Coordinates	SNP Bases by variety		
	Start	Stop				Anchor	Gemmiza HSP101	Sids HSP101
Anchor_1	1	600	4	0	217	A	A	G
					358	A	A	G
					389	A	A	G
					537	A	A	T
Anchor_3	601	1200	4	2	715	A	A	G
					764	A	A	G
					1020	A	A	G
					1040	A	A	G
Anchor_8	2101	2458	2	1	2283	A	A	G
					2294	A	A	T

Table (12): Summary of single nucleotide polymorphism (SNP) characterization at by SNPs Finder program.

SNPs	Gemmiza9 HSP101	%	Sids 12 HSP101	%
Transition	-	0%	8	66%
Tranversion	-	0%	2	17%
(InDel)	1	100%	2	17%
Frequency (bp/SNP)	-	-	180	-
Frequency (bp/InDel)	1795	-	898	-

AF174433.1_HSP101c	AGGCGCTCGCGGGCGGCGCACGAGATGGCGTCCGAGGCCGG	167
Gemmeiza9_HSP101	AGGCGCTCGCGGGCGGCGCACGAGATGGCGTCCGAGGCCGG	40
Sids12_HSp101	AGGCGCTCGCGGGCGGCGCACGAGATGGCGTCCGAGGCCGG	40
Consensus	aggcgctcgcggcggcgcacgagatggcgtccgaggccgg	
AF174433.1_HSP101c	CCACGCGCAGCTCACGCCGCTGCACCTCGCCGCGGGCGCTC	207
Gemmeiza9_HSP101	CCACGCGCAGCTCACGCCGCTGCACCTCGCCGCGGGCGCTC	80
Sids12_HSp101	CCACGCGCAGCTCACGCCGCTGCACCTCGCCGCGGGCGCTC	80
Consensus	ccacgcgcagctcacgccgctgcaacctcgcccgccggcgctc	
AF174433.1_HSP101c	GCGGCGGACAGGTTCGGGCATCCTCCGCCAGGCCATCGCCC	247
Gemmeiza9_HSP101	GCGGCGGACAGGTTCGGGCATCCTCCGCCAGGCCATCGCCC	120
Sids12_HSp101	GCGGCGGACAGGTTCGGGCATCCTCCGCCAGGCCATCGCCC	120
Consensus	gcgccggacaggtcgggcatcctccgccaggccatcgccc	
AF174433.1_HSP101c	ACGCGTCCGGCGGCAACGACGCCGCGGCCGAGTCGTTCGA	287
Gemmeiza9_HSP101	ACGCGTCCGGCGGCAACGACGCCGCGGCCGAGTCGTTCGA	160
Sids12_HSp101	ACGCGTCCGGCGGCAACGACGCCGCGGCCGAGTCGTTCGA	160
Consensus	acgcgtccggcggcaacgacgcccgccgagtcgttcga	
AF174433.1_HSP101c	GCGCGTCCGCTCCGCCGCGCTCAAGCGGCTGCCCTCGCAG	327
Gemmeiza9_HSP101	GCGCGTCCGCTCCGCCGCGCTCAAGCGGCTGCCCTCGCAG	200
Sids12_HSp101	GCGCGTCCGCTCCGCCGCGCTCAAGCGGCTGCCCTCGCAG	200
Consensus	gcgcgtccgctccgcccgctcaagcggctgccctcgcag	
AF174433.1_HSP101c	TCCCCGCCGCCGACACCGTCCCAGCCTCCACCTCGCTGG	367
Gemmeiza9_HSP101	TCCCCGCCGCCGACACCGTCCCAGCCTCCACCTCGCTGG	240
Sids12_HSp101	TCCCCGCCGCCGACACCGTCCCAGCCTCCACCTCGCTGG	240
Consensus	tccccgccgccgacaccgtcccagcctccacctcgctgg	
AF174433.1_HSP101c	TCAAGGCCGTCCGCCGCGCGCAGTCGGCGCAGAAGTCGCG	407
Gemmeiza9_HSP101	TCAAGGCCGTCCGCCGCGCGCAGTCGGCGCAGAAGTCGCG	280
Sids12_HSp101	TCAAGGCCGTCCGCCGCGCGCAGTCGGCGCAGAAGTCGCG	280
Consensus	tcaaggcgtccgcccgccgagtcggcgcagaaagtcgcg	
AF174433.1_HSP101c	CGGCGACTCGCACCTCGCCGTCGACCAGCTGCTCATGGGC	447
Gemmeiza9_HSP101	CGGCGACTCGCACCTCGCCGTCGACCAGCTGCTCATGGGC	320
Sids12_HSp101	CGGCGACTCGCACCTCGCCGTCGACCAGCTGCTCATGGGC	320
Consensus	cgcgactcgcacctcgccgtcgaccagctgctcatgggc	
AF174433.1_HSP101c	CTCCTCGAGGACCCGACAGATCTCCGACCGGCTCAAGGAGG	487
Gemmeiza9_HSP101	CTCCTCGAGGACCCGACAGATCTCCGACCGGCTCAAGGAGG	360
Sids12_HSp101	CTCCTCGAGGACCCGACAGATCTCCGACCGGCTCAAGGAGG	360
Consensus	ctcctcgaggaccgacagatctccgaccggctcaaggagg	
AF174433.1_HSP101c	CCGGCATCTCCGCTGCGCGGGTGAAGGCCGAGGTCGAGAA	527
Gemmeiza9_HSP101	CCGGCATCTCCGCTGCGCGGGTGAAGGCCGAGGTCGAGAA	400
Sids12_HSp101	CCGGCATCTCCGCTGCGCGGGTGAAGGCCGAGGTCGAGAA	400
Consensus	ccggcatctccgctgcgccgggtgaaggccgaggtcgagaa	
AF174433.1_HSP101c	GTCGCGGGAGGGCGACAACCGGCGCGTGGAGTCCGCGTCCG	567
Gemmeiza9_HSP101	GTCGCGGGAGGGCGACAACCGGCGCGTGGAGTCCGCGTCCG	440
Sids12_HSp101	GTCGCGGGAGGGCGACAACCGGCGCGTGGAGTCCGCGTCCG	440
Consensus	gctccgggaggggcgacaaccggcgcgaggagtcgcgctcg	
AF174433.1_HSP101c	GGGGACACCAACTTCCAGGCCCTCAAGACGTACGGCCCGCG	607
Gemmeiza9_HSP101	GGGGACACCAACTTCCAGGCCCTCAAGACGTACGGCCCGCG	480
Sids12_HSp101	GGGGACACCAACTTCCAGGCCCTCAAGACGTACGGCCCGCG	480
Consensus	ggggacaccaacttccaggccctcaagagctacggcccgcg	
AF174433.1_HSP101c	ACCTCGTGGAGGTGGCCGGCAAGCTGGACCCGGTTCATCGG	647
Gemmeiza9_HSP101	ACCTCGTGGAGGTGGCCGGCAAGCTGGACCCGGTTCATCGG	520
Sids12_HSp101	ACCTCGTGGAGGTGGCCGGCAAGCTGGACCCGGTTCATCGG	520
Consensus	acctcgtggaggtggccggcaagctggaccgggtcatcgg	
AF174433.1_HSP101c	CCGCGACGAGGAGATCCGGCGCGTGGTGCGGATCCTGTCCG	687
Gemmeiza9_HSP101	CCGCGACGAGGAGATCCGGCGCGTGGTGCGGATCCTGTCCG	560
Sids12_HSp101	CCGCGACGAGGAGATCCGGCGCGTGGTGCGGATCCTGTCCG	560
Consensus	cccgacgaggagatccggcgcggtggtgcggatcctgtccg	
AF174433.1_HSP101c	CGGCGCACAAAGAACAACCCCGTCTCTCCGCGAGCCCG	727
Gemmeiza9_HSP101	CGGCGCACAAAGAACAACCCCGTCTCTCCGCGAGCCCG	600
Sids12_HSp101	CGGCGCACAAAGAACAACCCCGTCTCTCCGCGAGCCCG	600
Consensus	cggcgcaaaagaacaaccctgctctccgagcccg	
AF174433.1_HSP101c	GCGTGGGCAAGACCCCGTGGTGGAGGGGCTCGCGCAGCG	767
Gemmeiza9_HSP101	GCGTGGGCAAGACCCCGTGGTGGAGGGGCTCGCGCAGCG	640
Sids12_HSp101	GCGTGGGCAAGACCCCGTGGTGGAGGGGCTCGCGCAGCG	640
Consensus	gcgtgggcaagaccctggtggaggggctcgcgcagcg	
AF174433.1_HSP101c	CGTCTGCGCGGCGACGTCCCCAGCAACCTCTGGACGT	806
Gemmeiza9_HSP101	CGTCTGCGCGGCGACGTCCCCAGCAACCTCTGGACGT	679
Sids12_HSp101	CGTCTGCGCGGCGACGTCCCCAGCAACCTCTGGACGT	679
Consensus	cgtctgcgccggcgacgtccccagcaacctctggacgt	

(A)

AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	GCGCCTGGTTCGCGCTCGACATGGGCGCGCTCGTGGCCGGC GCGCCTGGTTCGCGCTCGACATGGGCGCGCTCGTGGCCGGC GCGCCTGGTTCGCGCTCGACATGGGCGCGCTCGTGGCCGGC g c g c c t g g t c g c g e t c g a c a t g g g c g c g e t c g t g g c e g g c	846 719 719
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	GCCAAGTACCGCGGCGAGTTTCGAAGGAGCGGCTCAAAGCCG GCCAAGTACCGCGGCGAGTTTCGAAGGAGCGGCTCAAAGCCG GCCAAGTACCGCGGCGAGTTTCGAAGGAGCGGCTCAAAGCCG g c c a a g t a c c g c g g c g a g t t c g a g g a g c g g e t c a a g g c c g	886 758 758
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	TGCTCAAGGAGGTGGAGGAGGCCGAGGGGAAGGTGATACT TGCTCAAGGAGGTGGAGGAGGCCGAGGGGAAGGTGATACT TGCTCAAGGAGGTGGAGGAGGCCGAGGGGAAGGTGATACT t g c t c a a g g a g g t g g a g g a g g c c g a g g g g a a g g t g a t a c t	926 798 798
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	GTTTCATCGACGAGATACACCTGGTGTCTCGGCGCCGGGCGG GTTTCATCGACGAGATACACCTGGTGTCTCGGCGCCGGGCGG GTTTCATCGACGAGATACACCTGGTGTCTCGGCGCCGGGCGG g t t c a t c g a c g a g a t a c a c c t g g t g t c t c g g c g c c g g g c g g	966 838 838
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	ACGGAGGGGTCAATGGACGCGGCCAACCTGTTCAAGCCGA ACGGAGGGGTCAATGGACGCGGCCAACCTGTTCAAGCCGA ACGGAGGGGTCAATGGACGCGGCCAACCTGTTCAAGCCGA a c g g a g g g g t c a a t g g a c g c g g c c a a c c t g t t c a a g c c g a	1006 878 878
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	TGCTGGCGAGGGGGGAGCTCAGGTGCATTGGCGCGGACGAC TGCTGGCGAGGGGGGAGCTCAGGTGCATTGGCGCGGACGAC TGCTGGCGAGGGGGGCGGCTCAGGTGCATTGGCGCGGCGAC t g c t g g c g a g g g g g c a g e t c a g g t g c a t t g g c g c g a c g a c	1046 918 918
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	CGCATGTGGCGGTGTTCAACACTCTGCTCCAGGTCTCTGGA CGCATGTGGCGGTGTTCAACACTCTGCTCCAGGTCTCTGGA CGCATGTGGCGGTGTTCAACACTCTGCTCCAGGTCTCTGGA c g c a t g t g g c g g t g t t c a a c a c t c t g e t c c a g g t c e t g g a	2183 987 979
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	CGATGGGCGGTTGACCGACGGGCAAGGCAGGACGGTTGAT CGATGGGCGGTTGACCGACGGGCAAGGCAGGACGGTTGAT CGATGGGCGGTTGACCGACGGGCAAGGCAGGACGGTTGAT c g a t g g g c g g t t g a c c g a c g g g c a a g g c a g g a c g g t t g a t	2223 1027 1019
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	TTTAGGAACACGGTGATCATCATGACCTCAAACCTTGGCG TTTAGGAACACGGTGATCATCATGACCTCAAACCTTGGCG TTTAGGAACACGGTGATCATCATGACCTCAAACCTTGGCG t t t a g g a a c a c g g t g a t c a t c a t g a c c t c a a a c c t t g g c g	2263 1067 1059
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	CGGAGCACCTCCTCGCCGGAATGGTGGGCAATTTCGATGAA CGGAGCACCTCCTCGCCGGAATGGTGGGCAATTTCGATGAA CGGAGCACCTCCTCGCCGGGATGGTGGGCAATTTCGATGAA c g g a g c a c c t c e t c g c c g g a a t g g t g g g c a a t t c g a t g a a	2303 1107 1099
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	GGTTGCTCGTGATCTGGTCATGCAGGAGGTGAGGAGGCAT GGTTGCTCGTGATCTGGTCATGCAGGAGGTGAGGAGGCAT GGTTGCTCGTGATCTGGTCATGCAGGAGGTGAGGAGGCAT g g t t g e t c g t g a t c t g g t c a t g c a g g a g g t g a g g a g g c a t	2343 1147 1139
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	TTCCGCCCGGAGCTGCTGAACCGTCTGGACGAGATCGTCA TTCCGCCCGGAGCTGCTGAACCGTCTGGACGAGATCGTCA TTCCGCCCGGAGCTGCTGAACCGTCTGGACGAGATCGTCA t t c c g c c c g g a g c t g e t g a a c c g t c t g g a c g a g a t c g t c a	2383 1187 1179
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	TCTTCGACCCTCTGTTCGCATGAGCAGCTGCGGAAAGGTTCGC TCTTCGACCCTCTGTTCGCATGAGCAGCTGCGGAAAGGTTCGC TCTTCGACCCTCTGTTCGCATGAGCAGCTGCGGAAAGGTTCGC t c t t c g a c c c t c t g t c g c a t g a g c a g c t g c g g a a g g t t c g c	2423 1227 1218
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	TCCGCTTCAGATGAAAGATGTGGCAGTCCGTCTTGCCGAG TCCGCTTCAGATGAAAGATGTGGCAGTCCGTCTTGCCGAG TCCGCTTCAGATGAAAGATGTGGCAGTCCGTCTTGCCGAG t c g g e t t c a g a t g a a a g a t g t g g c a g t c c g t c t t g c c g a g	2463 1267 1258
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	AGGGGCGTTGCTCTGGCCGTCACCGACGCCGCCCTGGACG AGGGGCGTTGCTCTGGCCGTCACCGACGCCGCCCTGGACG AGGGGCGTTGCTCTGGCCGTCACCGACGCCGCCCTGGACG a g g g g c g t t g e t c t g g c c g t c a c c g a c g c c c c t g g a c g	2503 1307 1298
AF174433.1_HSP101c Gemmeiza9_HSP101 Sids12_HSp101 Consensus	TCATCCTGTCACTGTCTTACGATCCGGTCTATGGCGCCAG TCATCCTGTCACTGTCTTACGATCCGGTCTATGGCGCCAG TCATCCTGTCACTGTCTTACGATCCGGTCTATGGCGCCGG t c a t c e t g t c a c t g t c t t a c g a t c c g g t c t a t g g c g c c a g	2543 1347 1338

(B)

AF174433.1_HSP101c	GCCAATCCGGAGATGGATCGAGAAGAGGATAGTGACGGAG	2583
Gemmeiza9_HSP101	GCCAATCCGGAGATGGATCGAGAAGAGGATAGTGACGGAG	1387
Sids12_HSp101	GCCAATCCGGAGATGGATCGAGAAGAGGATAGTGACGGAG	1378
Consensus	gccaatccggagatggatcgagaagaggatagtgacggag	
AF174433.1_HSP101c	CTCTCCAAGATGTTGATCCGCGAGGAGATCGACGAGAACT	2623
Gemmeiza9_HSP101	CTCTCCAAGATGTTGATCCGCGAGGAGATCGACGAGAACT	1427
Sids12_HSp101	CTCTCCAAGATGTTGATCCGCGAGGAGATCGACGAGAACT	1418
Consensus	ctctccaagatgttgatccgcgaggagatcgacgagaact	
AF174433.1_HSP101c	CCACGGTGTACATCGACGCTGCGCCCAGCAAGGACGAGCT	2663
Gemmeiza9_HSP101	CCACGGTGTACATCGACGCTGCGCCCAGCAAGGACGAGCT	1467
Sids12_HSp101	CCACGGTGTACATCGACGCTGCGCCCAGCAAGGACGAGCT	1458
Consensus	ccacggtgtacatcgacgctgcgcccagcaaggacgagct	
AF174433.1_HSP101c	GACCTATGGCGTCGACAAGCACGGAGGGCTGGTGAACGCG	2703
Gemmeiza9_HSP101	GACCTATGGCGTCGACAAGCACGGAGGGCTGGTGAACGCG	1507
Sids12_HSp101	GACCTATGGCGTCGACAAGCACGGAGGGCTGGTGAACGCG	1498
Consensus	gacctatggcgtcgacaagcacggagggctggtgaacgcg	
AF174433.1_HSP101c	CGCACGGGCCACAAGTCCGACATCCTGATCCAGGTTCTTA	2743
Gemmeiza9_HSP101	CGCACGGGCCACAAGTCCGACATCCTGATCCAGGTTCTTA	1547
Sids12_HSp101	CGCACGGGCCACAAGTCCGACATCCTGATCCAGGTTCTTA	1538
Consensus	cgcacggggccacaagtccgacatcctgatccaggttctta	
AF174433.1_HSP101c	GCGGAGCTGTTGGGGGCGATGCGGCGCACGCCGTGAAGAA	2783
Gemmeiza9_HSP101	GCGGAGCTGTTGGGGGCGATGCGGCGCACGCCGTGAAGAA	1587
Sids12_HSp101	GCGGAGCTGTTGGGGGCGATGCGGCGCACGCCGTGAAGAA	1578
Consensus	gcgagctgttgggggcgatgcgcgccacgccgtgaagaa	
AF174433.1_HSP101c	GATGAGATCATGCAGGACAGCGGAGAGGTGGACGACATG	2823
Gemmeiza9_HSP101	GATGAGATCATGCAGGACAGCGGAGAGGTGGACGACATG	1627
Sids12_HSp101	GATGAGATCATGCAGGACAGCGGAGAGGTGGACGACGTTG	1618
Consensus	gatgaagatcatgcaggacagcggagaggtggacgacatg	
AF174433.1_HSP101c	GAGGAAGAGTAGATGGAAACCGCATCGATTGATCCCTTTC	2863
Gemmeiza9_HSP101	GAGGAAGAGTAGATGGAAACCGCATCGATTATCCCTTTC	1666
Sids12_HSp101	GAGGAAGAGTAGATGGAAACCGCATCGATTGATCCCTTTC	1658
Consensus	gaggaagagttagatggaaaccgcattgatccctttc	
AF174433.1_HSP101c	TGACTGTAGTTCCAAGTGCCTGCACCAGGCAGTCGTATGA	2903
Gemmeiza9_HSP101	TGACTGTAGTTCCAAGTGCCTGCACCAGGCAGTCGTATGA	1706
Sids12_HSp101	TGACTGTAGTTCCAAGTGCCTGCACCAGGCAGTCGTATGA	1698
Consensus	tgactgtagttccaagtgcctgcaccaggcagtcgtatga	
AF174433.1_HSP101c	GCTCTGTTTTTGCCTTTTGGACCGTGCTGATTTAGGTAGTT	2943
Gemmeiza9_HSP101	GCTCTGTTTTTGCCTTTTGGACCGTGCTGATTTAGGTAGTT	1746
Sids12_HSp101	GCTCTGTTTTTGCCTTTTGGACCGTGCTGATTTAGGTAGTT	1738
Consensus	gctctgtttttgccttttggaccgtgctgatttaggtagtt	
AF174433.1_HSP101c	CGGAATCGTATCTGTTGAATTGGGGGTGAAATGAATTGTA	2983
Gemmeiza9_HSP101	CGGAATCGTATCTGTTGAATTGGGGGTGAAATGAATTGTA	1786
Sids12_HSp101	CGGAATCGTATCTGTTGAATTGGGGGTGAAATGATTTGTA	1778
Consensus	cggaatcgtatctgttgaattgggggtgaaatgaattgta	

(C)

Figure (18): SNPs detection (A, B and C) for Anchor AF174433.1 (HSP101c) and 2near neighbors sids12 HSP101 and Gemmeiza9 HSP101 by using the DNAMAN® software (Lyon BioSoft, Quebec, Canada). Gaps in the sequences are indicated by dots “.” Indicate conserved consensus sequences at last sequence. Indicate SNPs by shading.

(A)

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201 GGCCTCCACCTCGCTGGTCAAGGCCGTCCGCCGCGCGCAAGTTCGGCGCAG AF174433.1 [1, 600]
201 GGCCTCCACCTCGCTGGTCAAGGCCGTCCGCCGCGCGCAAGTTCGGCGCAG Gemmeiza9 [1, 473]
201 GGCCTCCACCTCGCTGGTCAAGGCCGTCCGCCGCGCGCAAGTTCGGCGCAG Sids12 [1, 473]

351 GGCCTCCACCTCGCTGGTCAAGGCCGTCCGCCGCGCGCAAGTTCGGCGCAG AF174433.1 [1, 600]
351 GGCCTCCACCTCGCTGGTCAAGGCCGTCCGCCGCGCGCAAGTTCGGCGCAG Gemmeiza9 [1, 473]
351 GGCCTCCACCTCGCTGGTCAAGGCCGTCCGCCGCGCGCAAGTTCGGCGCAG Sids12 [1, 473]

501 TGCGCGGGTGAAGGCCGAGGTCGAGAAGCTCCGGGCAAGGCGACAACCGGC AF174433.1 [1, 600]
501 TGCGCGGGTGAAGGCCGAGGTCGAGAAGCTCCGGGCAAGGCGACAACCGGC Gemmeiza9 [1, 473]
501 TGCGCGGGTGAAGGCCGAGGTCGAGAAGCTCCGGGCAAGGCGACAACCGGC Sids12 [1, 473]

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(B)

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101 ACAACCCCGTCCTCATCGGCGAGCCCGGCGTGGGCAAGACCGCCGTG AF174433.1 [601, 1200]
101 ACAACCCCGTCCTCATCGGCGAGCCCGGCGTGGGCAAGACCGCCGTG Gemmeiza9 [474, 947]
101 ACAACCCCGTCCTCATCGGCGAGCCCGGCGTGGGCAAGACCGCCGTG Sids12 [474, 941]

151 GAGGGGCTCGCGCAAGCGCGTCGTGCGCGGCGACGTCCCCAGCAACCT AF174433.1 [601, 1200]
151 GAGGGGCTCGCGCAAGCGCGTCGTGCGCGGCGACGTCCCCAGCAACCT Gemmeiza9 [474, 947]
151 GAGGGGCTCGCGCAAGCGCGTCGTGCGCGGCGACGTCCCCAGCAACCT Sids12 [474, 941]

251 AGTACCGCGCGAGTTCGAGCAGCGGCTCAAGCCCGTGTCAAGGAG AF174433.1 [601, 1200]
251 AGTACCGCGCGAGTTCGAGCAGCGGCTCAAGCCCGTGTCAAGGAG Gemmeiza9 [474, 947]
251 AGTACCGCGCGAGTTCGAGCAGCGGCTCAAGCCCGTGTCAAGGAG Sids12 [474, 941]

401 AGCCGATGCTGGCGAGGGGGCAGGCTCAGGTGCATTGGCGCGCCGACGACC AF174433.1 [601, 1200]
401 AGCCGATGCTGGCGAGGGGGCAGGCTCAGGTGCATTGGCGCGCCGACGACC Gemmeiza9 [474, 947]
401 AGCCGATGCTGGCGAGGGGGCAGGCTCAGGTGCATTGGCGCGCCGACGACC Sids12 [474, 941]

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(C)

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151 TCAAACCTTGGCGCGGAGCACCTCCTCGCCGGAATGGTGGGCAATT AF174433.1 [2101, 2458]
151 TCAAACCTTGGCGCGGAGCACCTCCTCGCCGGAATGGTGGGCAATT Gemmeiza9 [948, 1262]
151 TCAAACCTTGGCGCGGAGCACCTCCTCGCCGGAATGGTGGGCAATT Sids12 [940, 1253]

301 CATGAGCAGCTGCGGAAAGGTCGCTCGGCTTCAGATGAAAGATGTGG AF174433.1 [2101, 2458]
301 CATGAGCAGCTGCGGAAAGGTCGCTCGGCTTCAGATGAAAGATGTGG Gemmeiza9 [948, 1262]
301 CATGAGCAGCTGCGGAAAGGTCGCTCGGCTTCAGATGAAAGATGTGG Sids12 [940, 1253]

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Figure (19): SNPs detection by Alignment of the sequences of Anchor AF174433.1 (*Triticum aestivum* heat shock protein 101 (HSP101c) mRNA complete cds) and 2near neighbors sids12 HSP101 and Gemmeiza9 HSP101 cDNAs. By using SNPs Finder web site (<http://snpsfinder.lanl.gov>).

(A): SNPs and INDELS at Anchor_1 Sequence Fragment.

(B): SNPs and INDELS at Anchor_3 Sequence Fragment.

(C): SNPs and INDELS at Anchor_8 Sequence Fragment.

Gaps in the sequences are indicated by dashes (-). Boxes indicate SNPs and INDELS.

4.4.2.2.HSP101 Amino acids composition changes in relation to tolerant and intolerant wheat

Proteins are a highly temperature-sensitive component of organisms and clear patterns of adaptive variation have been discovered in structural and functional properties of proteins from species adapted to different temperatures (Somero, 2004). With the advent of high-resolution techniques in molecular and structural biology, it is now possible to determine the amount of change in amino acid sequence needed for adaptation, the locations of these changes in the protein's three-dimensional structure and the mechanisms by which substitutions influence stability and kinetic properties.

Alignment of the deduced amino acid sequences of *TaHSP101c*, Gemmeiza9 HSP101 and obtained with DNAMAN® software (Lyon BioSoft, Quebec, Canada) (Fig. 20) showed that *Sids12_HSP101* shares 97.9% identity with *AF174433.1(HSP101c)* and *Gemmeiza9 HSP101* share 99.5% identity with *AF174433.1(HSP101c)* (Tabassum Jehan and Suman, 2006). Mutations in coding regions of genes, leading to amino acid substitutions, tend to alter the structure of the encoded protein. Some of the changes are conservative, i.e. have minimal impact on the protein structure, while some are radical and may lead to severe changes in its properties (Win J., et al., 2011).

Detection of amino acids changes by Mega 6.06 program revealed no effective changes at *Gemmeiza9_HSP101*, while, there are ten amino acid position change in *Sids12_HSP101* (Table 12). Win et al., 2011 indicated that, a single amino acid replacement is sufficient to adapt a protein to a new thermal range. The probability of substitution of one amino acid by another depends on the structure of the genetic code (i.e. on the number of mutations necessary to pass from one codon to another) and also on the phenotypic effect of that mutation (Higgins 2000).

Moreover calculation of relative precentage change of amino acids frequencies at sequences of *Sids12_HSP101* to *AF174433.1 TdHSP101c* revealed 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 (Table 14 and 15). Substitutions of one amino acid by another with similar biochemical properties generally do not greatly affect the structure and hence the function of the protein (Higgins 2000). Jehan, & Lakhanpaul, (2006) indicated that SNPs in coding regions may have functional significance if the resulting amino acid change causes the altered phenotype. SNP markers associated with phenotypic changes pinpoint functional polymorphism.

It is important to note that the probability of substitution of one amino acid by another depends on the evolutionary distance between sequences. The deduced HSP101 amino acid sequences were compared to the known HSP101 isoformes. Analyses of Evolutionary Divergence between sequences *AF174433.1 (HSP101c)*, *AF083344.2 (HSP101)*, *Sids12 HSp101* and *Gemmeiza9 HSP101* were conducted using the Maximum Composite Likelihood model (MEGA6). As shown in (Figure 21) and (Table 16) *Sids12HSp101* 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 distance between *AF174433.1 (HSP101c)* and

Sids12_HSp101 was 0.01 %. These results confirmed by our previous data which indicated that *Sids12_HSP101* contained ten amino acid position changes in comparison with AF174433.1 (HSP101c), with no amino acids position change at Gemmeiza9 HSP101.

Higgins (2000) suggested that at short evolutionary distances, probabilities of substitution mainly reflect the structure of the genetic code, whereas at larger distances, probabilities of substitution depend essentially on biochemical similarities between amino acids.

AF174433.1 HSP101	RRSRRRTRVWRPRPATRSSRRCTSPRRSRRTRGRASSARPSP	40
Gemmeiza9_HSP101	RRSRRRTRVWRPRPATRSSRRCTSPRRSRRTRGRASSARPSP	40
Sids12_HSp101	RRSRRRTRVWRPRPATRSSRRCTSPRRSRRTRGRASSARPSP	40
Consensus	r r s r r r t r w r p r p a t r s s r r c t s p r r s r r t g r a s s a r p s p	
AF174433.1 HSP101	TRPAATTPRPSRSSASRPPRSSGCPRSPRRPTPSRPPPRW	80
Gemmeiza9_HSP101	TRPAATTPRPSRSSASRPPRSSGCPRSPRRPTPSRPPPRW	80
Sids12_HSp101	TRPAATTPRPSRSSASRPPRSSGCPRSPRRPTPSRPPPRW	80
Consensus	t r p a a t t p r p s r s s a s r p p r s s g c p r s p r r p t p s r p p p r w	
AF174433.1 HSP101	SRPSAARSRRRSRAATRTPSTSCSVASSRTRRSPTRSRR	120
Gemmeiza9_HSP101	SRPSAARSRRRSRAATRTPSTSCSVASSRTRRSPTRSRR	120
Sids12_HSp101	SRPSAARSRRRSRAATRTPSTSCSVASSRTRRSPTRSRR	120
Consensus	s r p s a a r s r r r s r a a t r t s p s t s c s v a s s r t r r s p t r s r r	
AF174433.1 HSP101	PASPLRGRPRSRSSG[E]ATTGAWSPRRGTPTSRPSRRTAAT	160
Gemmeiza9_HSP101	PASPLRGRPRSRSSG[E]ATTGAWSPRRGTPTSRPSRRTAAT	160
Sids12_HSp101	PASPLRGRPRSRSSG[V]ATTGAWSPRRGTPTSRPSRRTAAT	160
Consensus	p a s p l r g r p r s r s s g e a t t g a w s p r r g t p t s r p s r r t a a t	
AF174433.1 HSP101	SVRWRASWTRSSAATRRSGAVCGSCRGAQRTPSSASPA	200
Gemmeiza9_HSP101	SVRWRASWTRSSAATRRSGAVCGSCRGAQRTPSSASPA	200
Sids12_HSp101	SVRWRASWTRSSAATRRSGAVCGSCRGAQRTPSSASPA	200
Consensus	s w r w r a s w t r s s a a t r r s g a v c g s c r g a q r t p s s a s p a	
AF174433.1 HSP101	WARPPWWRGSRSSASCAATSPATSWTCAVSRSTWARSWPAP	240
Gemmeiza9_HSP101	WARPPWWRGSRSSASCAATSPATSWTCAVSRSTWARSWPAP	240
Sids12_HSp101	WARPPWWRGSRSSASCAATSPATSWTCAVSRSTWARSWPAP	240
Consensus	w a r p p w w r g s r s s a s c a a t s p a t s w t c a v s r s t w a r s w p a p	
AF174433.1 HSP101	STAASSRSGSRPCSRWRPRGRYCSSTRYTWCSAPGGRR	280
Gemmeiza9_HSP101	STAASSRSGSRPCSRWRPRGRYCSSTRYTWCSAPGGRR	279
Sids12_HSp101	STAASSRSGSRPCSRWRPRGRYCSSTRYTWCSAPGGRR	279
Consensus	s t a a s s r s g s r p c s r r w r p r g r y c s s t r y t w c s a p g g r r	
AF174433.1 HSP101	GQWTRPTCSSRCVRGGS[S]GALARRPSRSTGTS[RR]MWRCS	320
Gemmeiza9_HSP101	GQWTRPTCSSRCVRGGS[S]GALARRPSRSTGTS[RR]MWRCS	319
Sids12_HSp101	GQWTRPTCSSRCVRGGS[S]GALARRPSRSTGTS[RR]MWRCS	316
Consensus	g q w t r p t c s s r c v r g g s s g a l a r r p s r s t g t s r r m w r c s	
AF174433.1 HSP101	TLCRSRWTMGGPTGKAGRLI LGTRSPQTLARSTSSPEVW	360
Gemmeiza9_HSP101	TLCRSRWTMGGPTGKAGRLI LGTRSPQTLARSTSSPEVW	359
Sids12_HSp101	TLCRSRWTMGGPTGKAGRLI LGTRSPQTLARSTSSPEVW	356
Consensus	t l c r s r w t m g g p t g k a g r l i l g t r s p q t l a r s t s s p e v w	
AF174433.1 HSP101	AIRRLLLVIVSCRRGGI[SARSCTVWTRSSSSSTLCRMS SCCR	400
Gemmeiza9_HSP101	AIRRLLLVIVSCRRGGI[SARSCTVWTRSSSSSTLCRMS SCCR	399
Sids12_HSp101	AIRRLLLVIVSCRRGGI[SARSCTVWTRSSSSSTLCRMS SCCR	395
Consensus	a i r r l l v i v s c r r g g i s a r s c t v w t r s s s s t l c r m s s c c r	
AF174433.1 HSP101	SLGFRKNWQSVLPRGALLWPSPTPPWTSSCHCLTI[RS]MAP	440
Gemmeiza9_HSP101	SLGFRKNWQSVLPRGALLWPSPTPPWTSSCHCLTI[RS]MAP	439
Sids12_HSp101	SLGFRKNWQSVLPRGALLWPSPTPPWTSSCHCLTI[RS]MAP	435
Consensus	s l g f r k n w q s v l p r g a l l w p s p t p p w t s s c h c l t i r s m a p	
AF174433.1 HSP101	GQSGDGSRRGRS[SPRCSARRSTRTPRCTSTLR]PARTSPMA	480
Gemmeiza9_HSP101	GQSGDGSRRGRS[SPRCSARRSTRTPRCTSTLR]PARTSPMA	479
Sids12_HSp101	GQSGDGSRRGRS[SPRCSARRSTRTPRCTSTLR]PARTSPMA	475
Consensus	g q s g d g s r r g r s s p r c s a r r s t r t p r c t s t l r p a r t s p m a	
AF174433.1 HSP101	STSTEGWTRARATSPTSSRFLAELLGAMRRTPRRRS[CR]TA	520
Gemmeiza9_HSP101	STSTEGWTRARATSPTSSRFLAELLGAMRRTPRRRS[CR]TA	519
Sids12_HSp101	STSTEGWTRARATSPTSSRFLAELLGAMRRTPRRCR[CR]TA	515
Consensus	s t s t e g w t r a r a t s p t s s r f l a e l l g a m r r t p r r r s c r t a	
AF174433.1 HSP101	ERWTTWR[.KSRWKPHR]I PFLFQVPAPGSRMSSVF[AF]GPC	559
Gemmeiza9_HSP101	ERWTTWR[.KSRWKPHR]I PFLFQVPAPGSRMSSVF[AF]GPC	557
Sids12_HSp101	AERWTTV[GKSRWKPHR]I PFLFQVPAPGSRMSSVF[AF]GPC	555
Consensus	e r w t t w r . k s r w k p h r i p f l f q v p a p g s r m s s v f a f g p c	
AF174433.1 HSP101	FRFGI VSVELGVKI[VVEDGK]KKK	582
Gemmeiza9_HSP101	FRFGI VSVELGVKI[VVEDGK]KKK	580
Sids12_HSp101	FRFGI VSVELGVK[VVEDGK]...	575
Consensus	f r f g i v s v e l g v k i v v e d g k k k k	

Figure (20): Alignment of the deduced amino acid sequences of TdHSP101c, Gemmeiza9 HSP101 and Sids12 HSP101 obtained with DNAMAN® software (Lyon BioSoft, Quebec, Canada). Gaps in the sequences are indicated by dots “.” Indicate conserved consensus sequences at last sequence. Indicate variance amino acids sequence by shading.

Table (13): Detection of amino acid position change by Mega 6.06 program

Amino acid position change	<i>AF174433.1(HSP101c)</i>	<i>Sids12_HSp101</i>	<i>Gemmeiza9_HSP101</i>
88	Serine	Glycine	Serine
137	Glutamic acid	Valine	Glutamic acid
213	Serine	Glycine	Serine
299	Serine	Glycine	Serine
363	Glutamic acid	Glycine	Glutamic acid
367	Isoleucine	Phenylalanine	Isoleucine
444	Isoleucine	Methionine	Isoleucine
531	NO	Cysteine	NO
544	Arginine	Glycine	Arginine
595	Isoleucine	Phenylalanine	Isoleucine

Table (14): Amino acids frequencies % at sequences of *AF174433.1 TdHSP101c*, *Gemmeiza9_HSP101* and *Sids12_HSP101*

Amino Acids											
Sequence Aname	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met
AF174433.1(HSP101c)	8.70	4.01	0.33	1.17	1.34	6.69	0.33	1.34	4.18	3.51	1.34
Gemmeiza9_HSP101	8.97	4.14	0.34	1.21	1.38	6.90	0.34	1.38	1.55	3.45	1.38
Sids12_HSp101	9.04	4.35	0.35	0.87	1.74	7.83	0.35	0.87	1.04	3.65	1.57
	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr	Total	
AF174433.1(HSP101c)	0.00	9.70	1.00	20.23	18.73	10.03	1.67	5.35	0.33	598	
Gemmeiza9_HSP101	0.00	10.00	1.03	20.69	19.31	10.34	1.72	5.52	0.34	580	
Sids12_HSp101	0.00	10.09	1.04	20.17	18.78	10.43	1.91	5.57	0.35	575	

Table (15): *Gemmeiza9_HSP101* and *Sids12_HSP101* Amino acids Relative change % to AF174433.1 (HSP101c)

Amino acid	% Relative change to AF174433.1(HSP101c)	
	Gemmeiza9_HSP101	Sids12_HSp101
Ala	3.10	3.90
Cys	3.20	8.40
Asp	3.00	6.00
Glu	3.40	-25.60
Phe	2.90	29.80
Gly	3.10	17.00
His	3.00	6.00
Ile	2.90	-35.00
Lys	-62.90	-75.11
Leu	-1.70	39.00
Met	2.98	17.00
Pro	3.10	4.00
Gln	3.00	4.00
Arg	2.27	- 0.29
Ser	3.10	0.26
Thr	3.10	3.90
Val	2.99	14.37
Trp	3.17	4.11
Tyr	3.00	6.00
Average	6.10	13.92

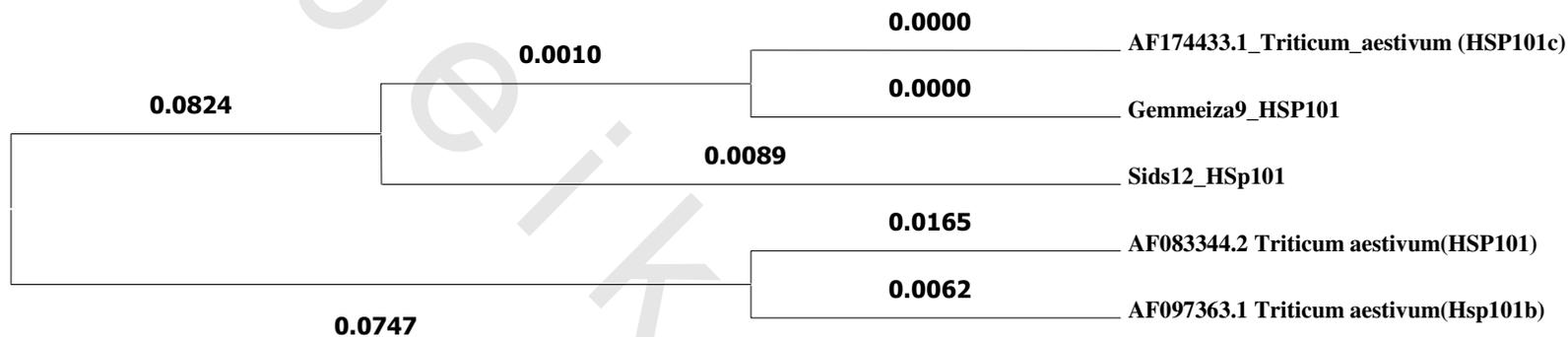


Figure (21): Dendrogram of genetic distance based on the bread wheat Hsp101 gene single nucleotide polymorphism markers constructed using MEGA6.06

Table (16): Genetic distance among AF174433.1 (HSP101c), AF083344.2 (HSP101), Sids12_HSp101 and Gemmeiza9_HSP101 using MEGA6.06

	AF174433.1 (HSP101c)	AF083344.2 (HSP101)	Sids12_HSp101	Gemmeiza9_HSP101
AF174433.1(HSP101c)	0.175			
AF083344.2 (HSP101)	0.165	0.023		
Sids12HSp101	0.010	0.183	0.172	
Gemmeiza9_HSP101	0.000	0.175	0.165	0.010