CHAPTER 4

RESULTS AND DISCUSSION

4.1 Physiological studies

4.1.1 Responses for membrane thermostabilityin 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 varities is shown in (Fig. 2). Results showed that the highest membrane injury was observed in cultivar Misr1 which was closely followed by varities 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 then 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 **Renu*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.

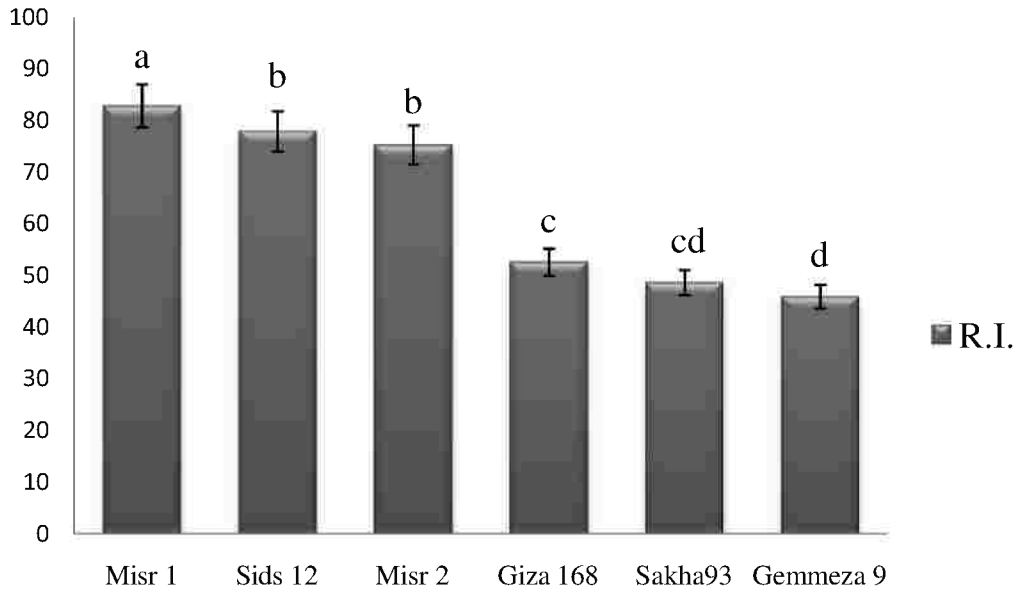


Figure (2): Membrane injury to high temperature of ten days old seedling of six wheat cultivars measured. Values followed by the different letter(s) are significantly different from each other by L.S.D. at 0.05.

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

varieties [A]	**Relative injury (%)**		
	At 25 □ c	**At 35 □ c**	**Calibrated** [y]
Misr1	14.69 ±0.434	86.12 ±0.956	82.85[a]
Sids12	16.09 ±0.390	72.88 ±1.310	77.91[b]
Misr2	15.64 ±0.228	74.71 ±0.921	75.246[b]
Giza168	17.82 ±0.422	32.96 ±0.826	52.57[c]
Sakha93	15.65 ±0.911	33.03 ±0.642	48.63[cd]
Gemmeiza9	18.45 ±0.217	35.93 ±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 cultiva (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.

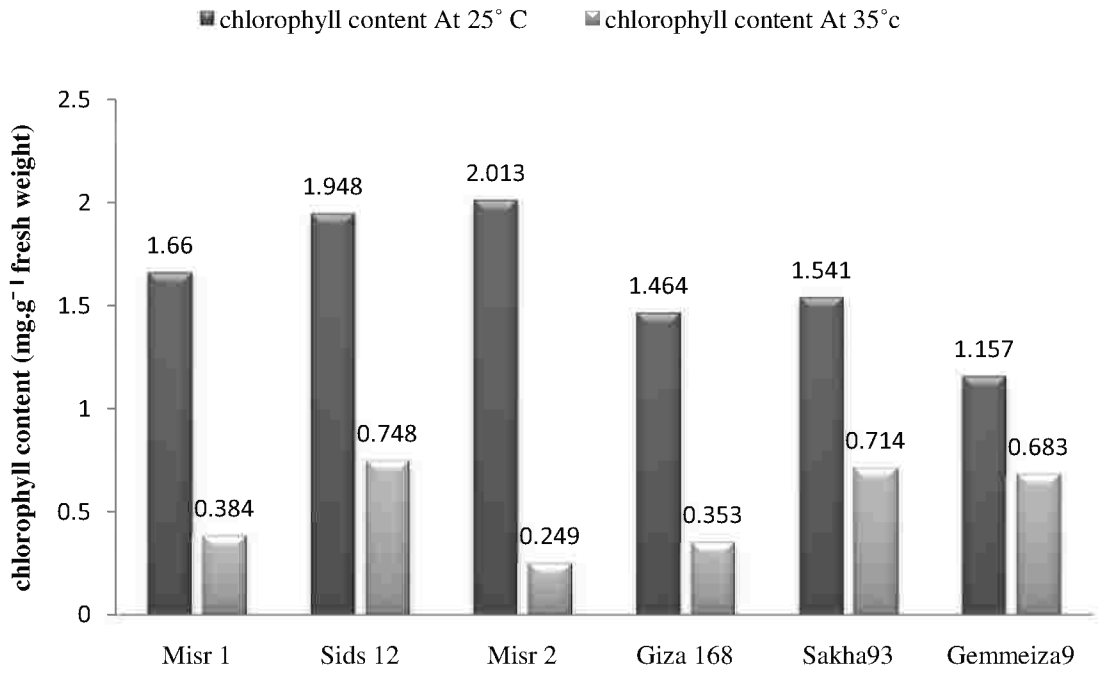


Figure (3): Average seedling chlorophyll content (mg.g □ fresh weight) at two temperature regimes for six Egyptian wheat varieties.

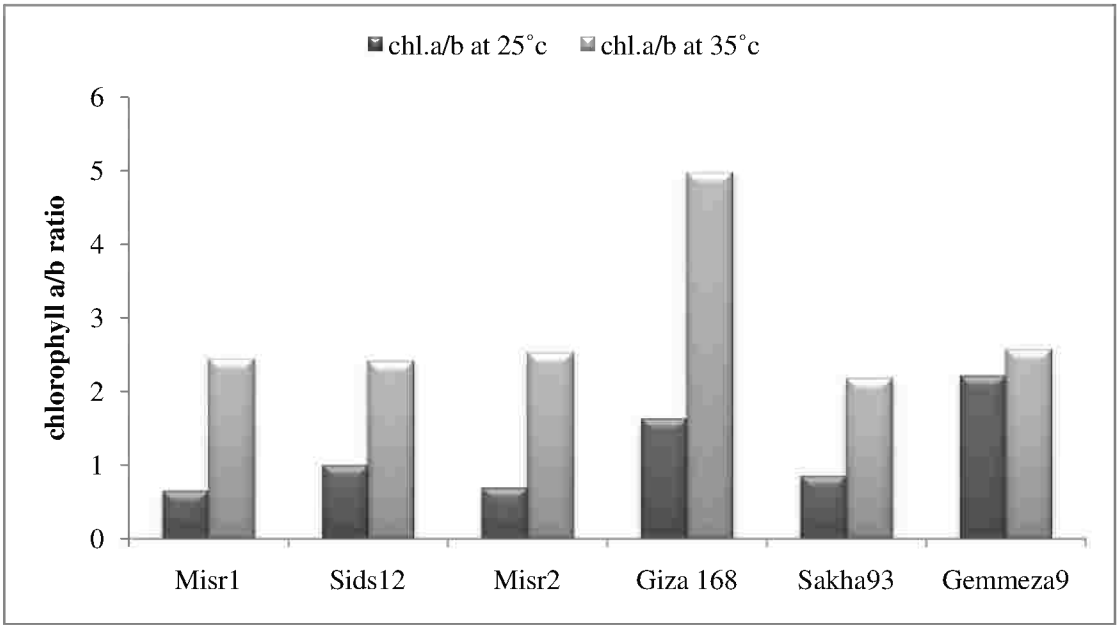


Figure (4): Average seedling chlorophyll *a/b* ratio under the effect of two temperature regimes for six Egyptian wheat varieties.

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[e]			
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 *a/b* 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.

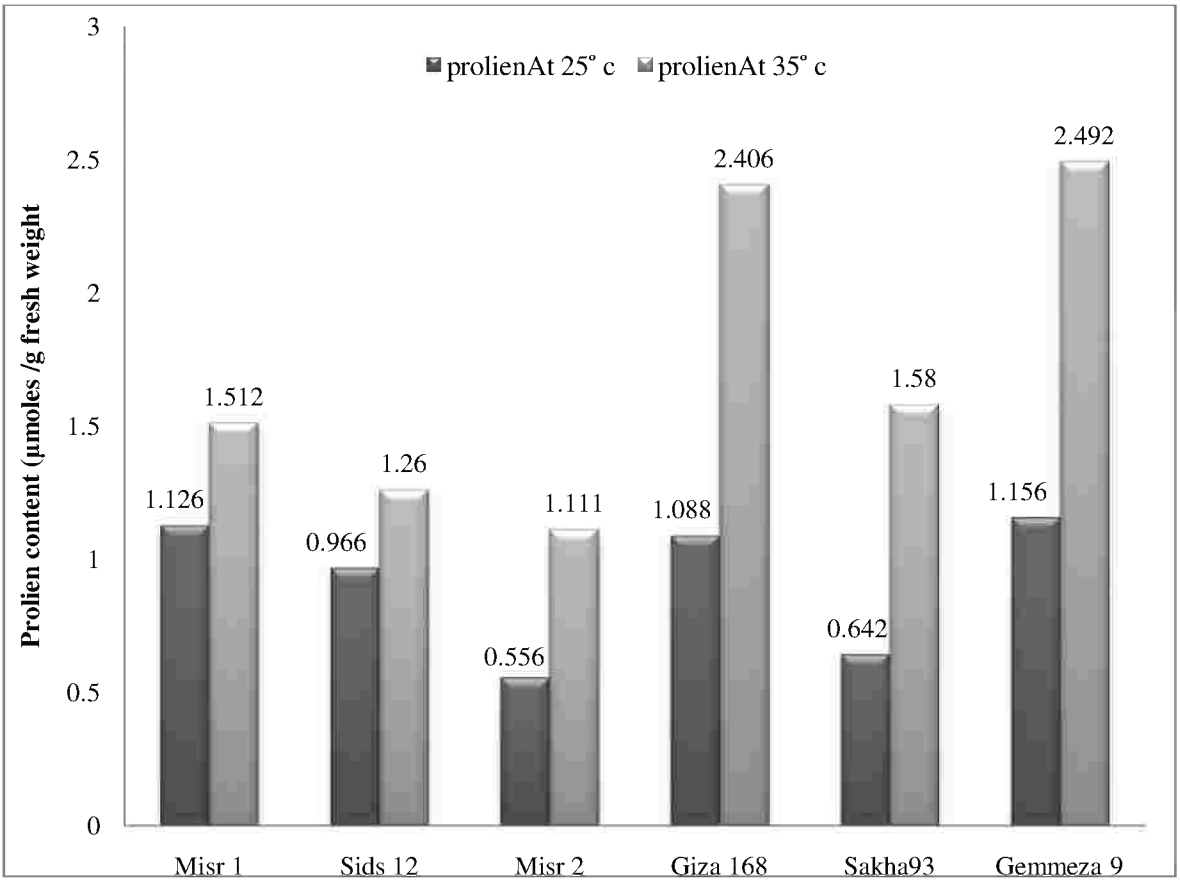


Figure (5): Average seedling proline content (µmoles/g fresh weight) at two temperature regimes for six Egyptian wheat varities.

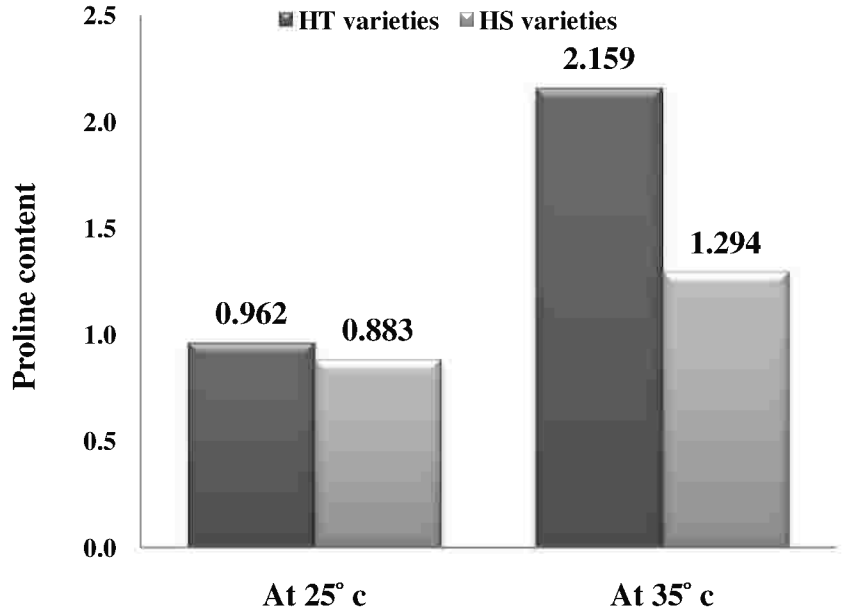


Figure (6): Average seedling proline content (µmoles/g fresh weight) of heat tolerant and heat sensitive wheat varieties as influenced by temperature regimes.

Table (6) Effect of heat stress treatment on Seedling Proline content (µmoles /g fresh weight) for six wheat varities (Mean ± SE)

Varities [A]	Seedling Proline content (µmoles /g fresh weight)				Significance		
	At 25° C	At 35° C	Relative to 25°C (%)	Mean	[A]	[B]	[AxB]
Misr 1	1.126^{g}±0.058	1.512^{d} ± 0.036	134	1.319c			
Sids 12	0.966^{j} ± 0.012	1.26^{e} ± 0.014	130	1.111d			
Misr 2	0.556^{l}± 0.019	1.111^{h}± 0.061	199	0.833e			
Giza 168	1.088^{i} ± 0.053	2.406^{c} ± 0.009	221	1.747b	**	**	**
Sakha93	0.642^{k} ±0.018	1.58^{c} ± 0.065	246	1.113d			
Gemmeiza9	1.156^{f} ± 0.024	2.492^{a} ± 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)** reveled 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 varities (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 varities (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 varities 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 varities compared to heat tolerant varities. In contrast, proline content and chlorophyll content were increased by 67.8% and 57% respectively in wheat heat tolerant varities compared to heat sensitive varities (Table 7).

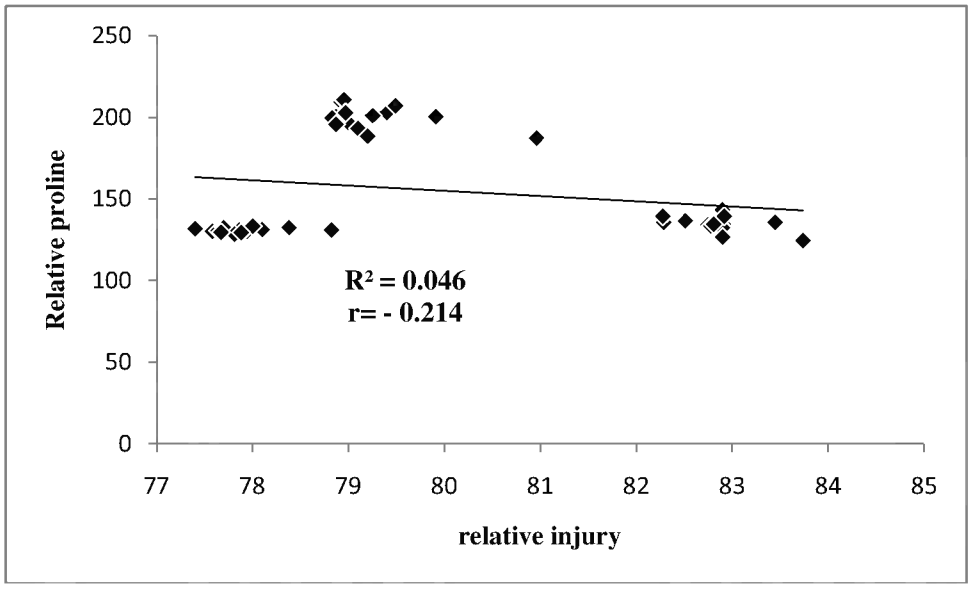


Figure (7): Relationship (r = - 0.214) between relative membrane injury and relative seedling proline content in heat sensitive wheat varities**.**

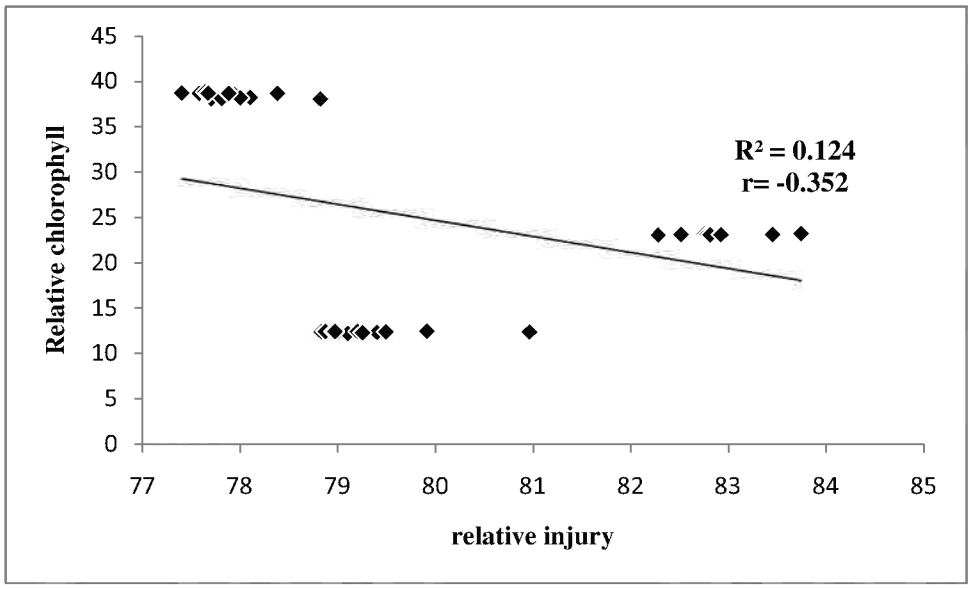


Figure (8): Relationship (r = - 0.352) between relative membrane injury and relative chlorophyll content in heat sensitive wheat varities**.**

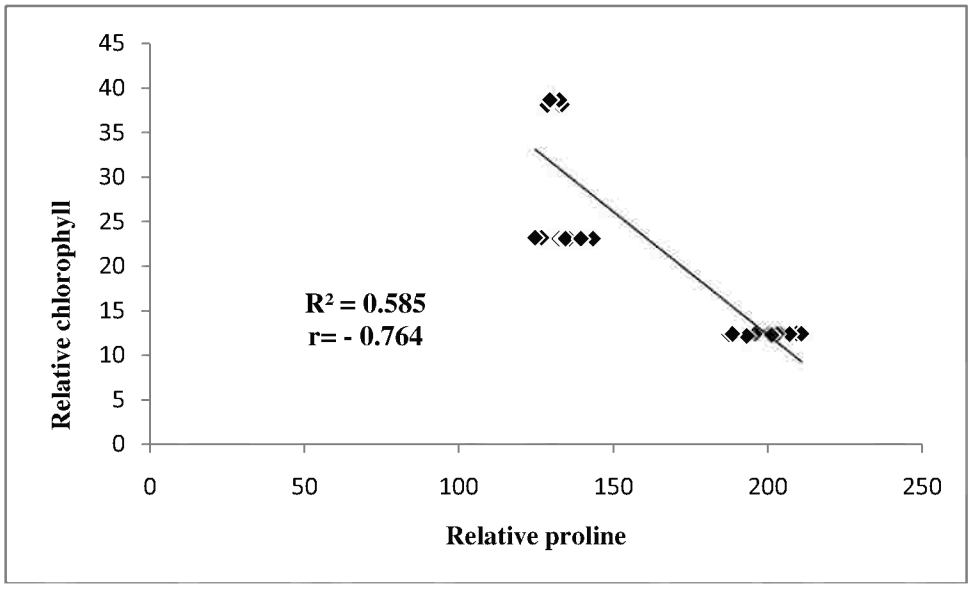


Figure (9): Relationship (r = - 0.764) between relative seedling proline content and relative chlorophyll content in heat sensitive wheat varities.

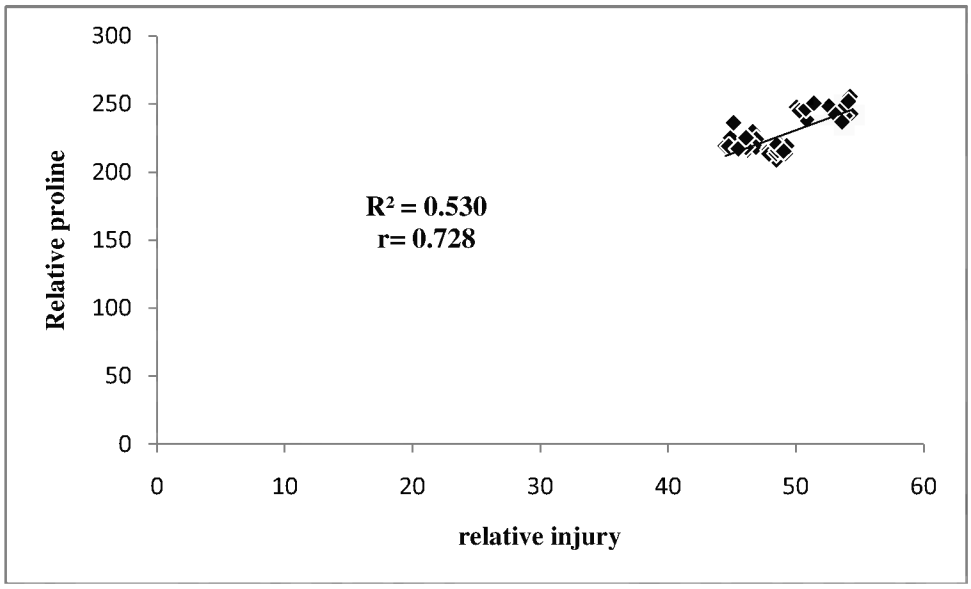


Figure (10): Relationship (r = 0.728) between relative membrane injury and relative seedling proline content in heat tolerant wheat varities.

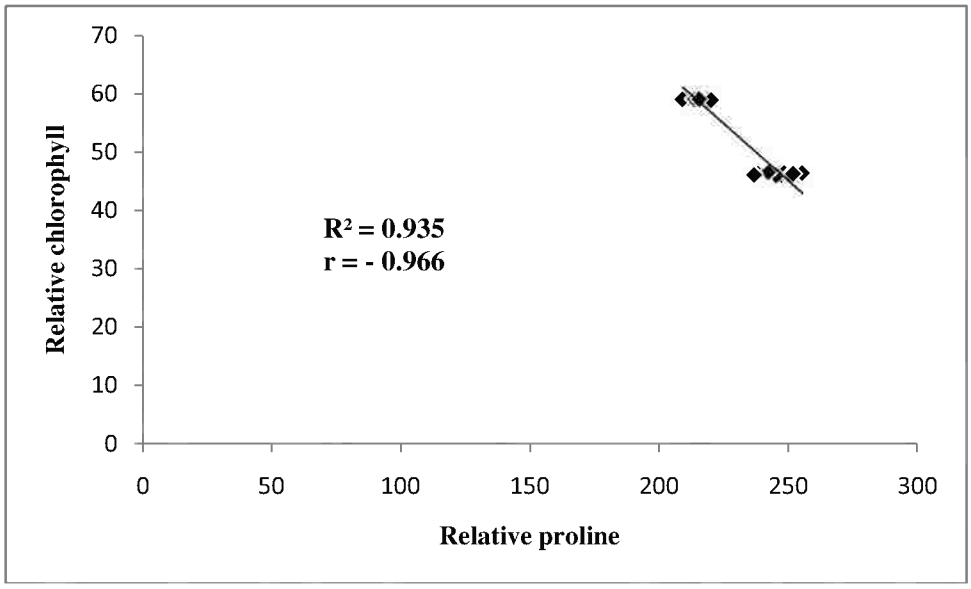


Figure (11): Relationship (r = - 0.966) between relative seedling proline content and relative chlorophyll content in heat tolerant wheat varities.

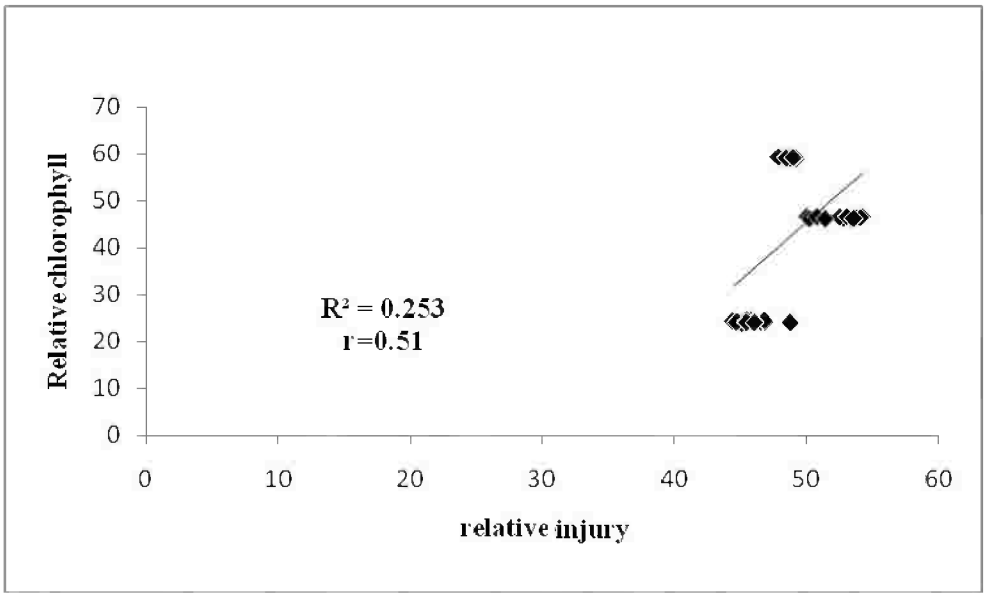


Figure (12): Relationship (r = 0.51) between relative membrane injury and relative chlorophyll content in heat tolerant wheat varities.

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

Measurment	Heat Senstive varities			Mean	Heat tolerant varities			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 varities control and treatment by heat until 14 days after germination **(Figure13)**. 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 plays 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 varitey '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 varities (**Table 8**) heat-tolerant varitey sakha93 which was closely followed by varities 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, varity Giza168 did not show *HSP101*high transcripts under this heat stress treatment (**Figure 14; A**). Also, in this study, heat-tolerant varity Gemmeiza9 which was followed by varity 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, varity 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 varities 'Gemmeiza9 and Misr1' but not in heat sensitive cutivar'Sids12'. Since HSP101 is essential for thermotolerance, heat tolerance of varities '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 HSP101with 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

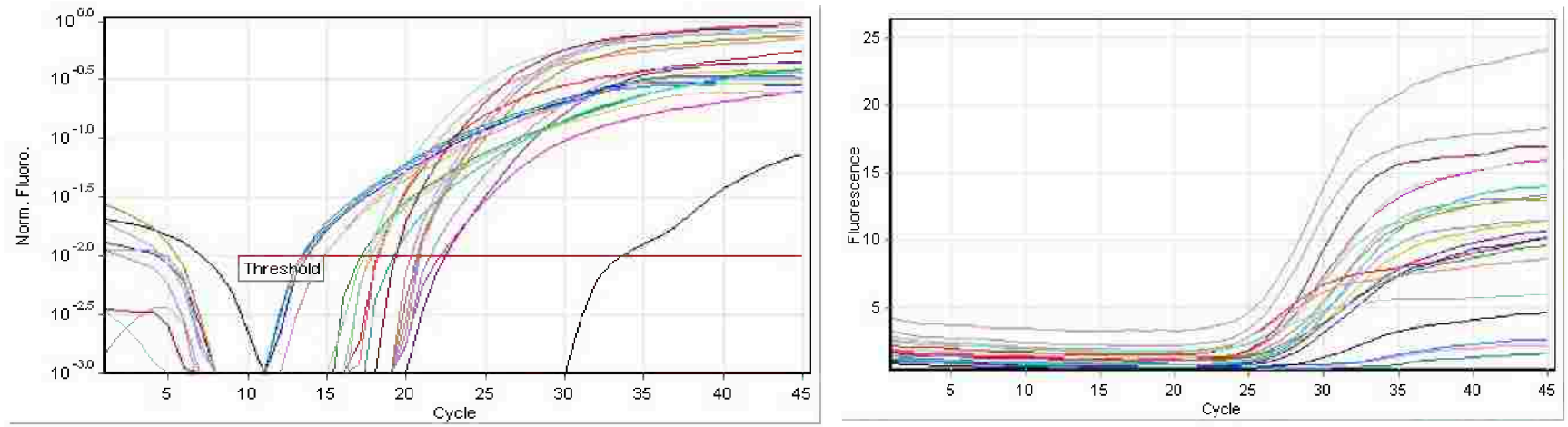
expression of HSP101 requires adaptation of wheat seedlings before treatment (**Fig.15**). **Moaed *et al.*, (2012)** showed that maintenance of high expression levels of *HSP101* during long-term heat stress in 'C306' may also be reason for better tolerance of 'C306' as compared with 'PBW343', as HSP101 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	ΔΔCT	$2^{-\Delta\Delta C}{}_T$ 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	-.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)

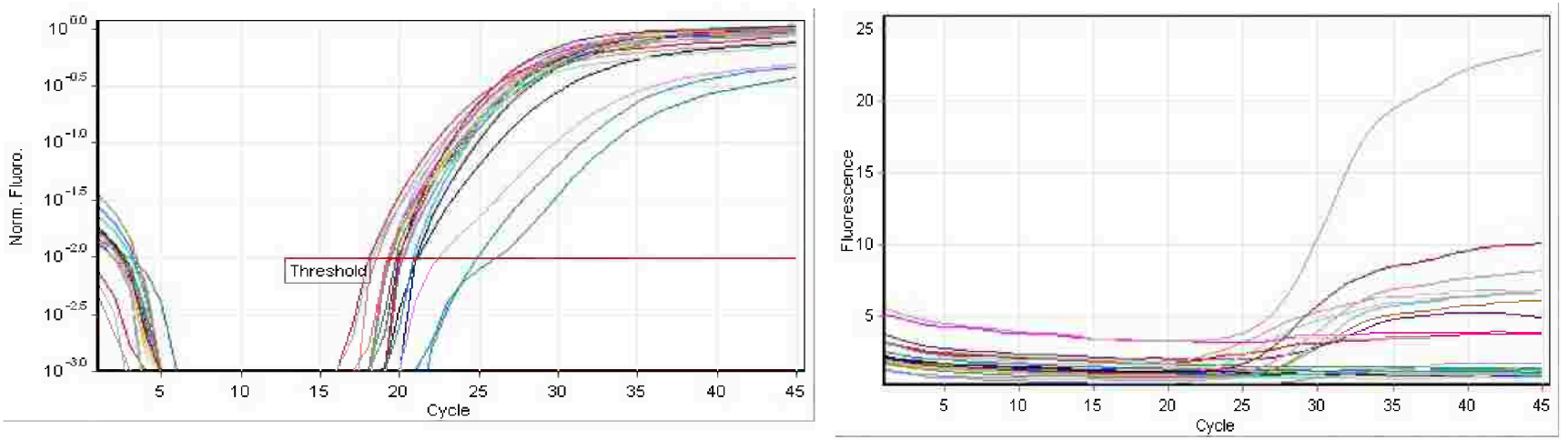
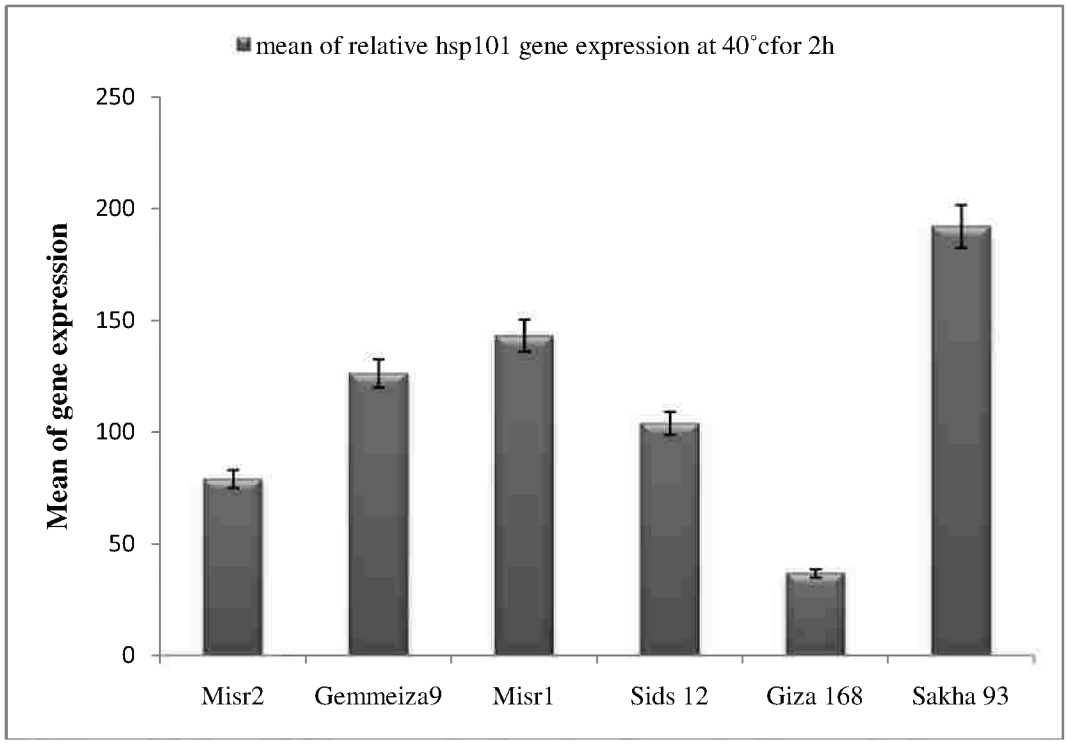
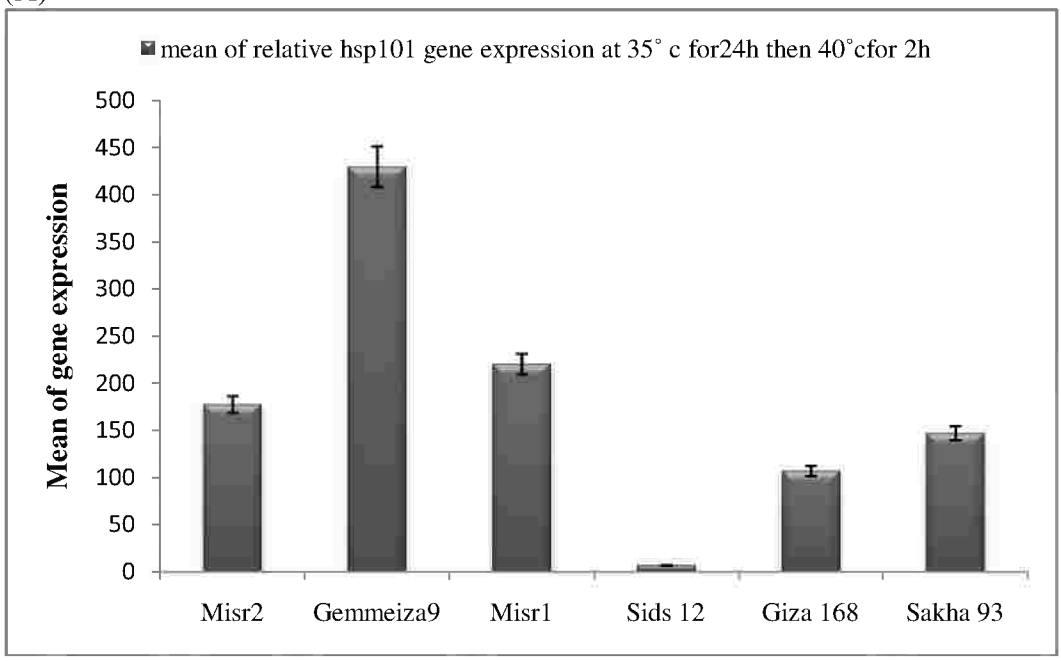


Figure (13): Quantification data by using HSP101 F3+R3 primers for HSP101 gene expression with β-Actin reference gene for RT-PCR cycling with SYBER-Green (A+B).



(A)



(B)

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

A. Mean of relative gene expression of HSp101 at six varities treated at 40°c for 2h.
B. Mean of relative gene expression of HSp101 at six varities treated at 34°C for 24 h + 2h at 42°C.

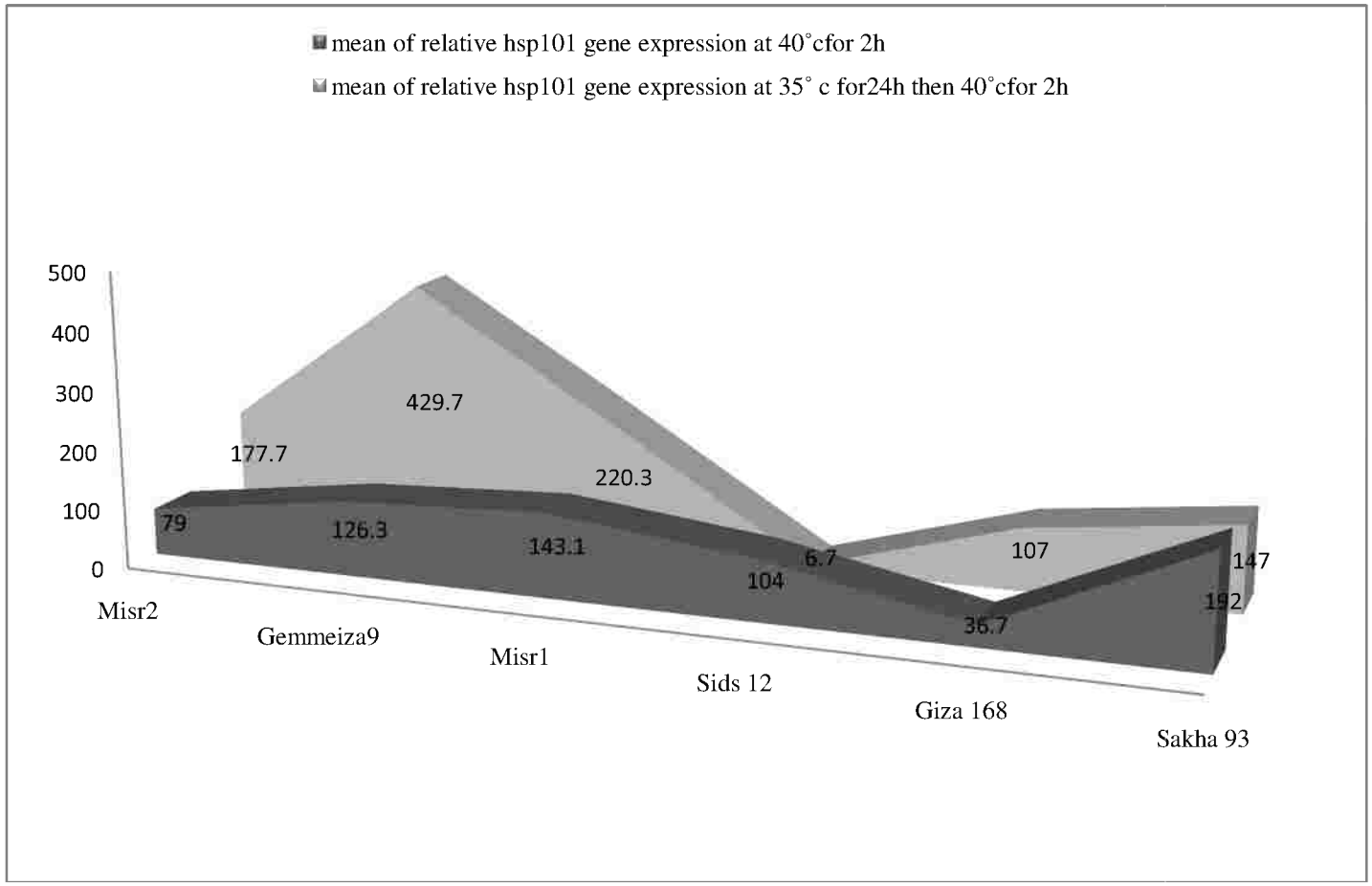


Figure (15): Relationship between HSp101gene expression level and heat treatment.

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_Gemmieza9*) 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,* Transtion 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_Gemmieza9*) 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_Gemmieza9* 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, transtions 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
CGCTGCACCTCGCCGCGGCGCTCGCGGCGGACGGGTCGGGCATCCTCCGCCAGGCCAT
CGCCCACGCGTCCGGCGGCAACGACGCCGCGGCCGAGTCGTTCGAGCGCGTCGCGTCC
GCCGCGCTCAAGCGGCTGCCCTCGCAGTCCCCGCCGCCCGACACCGTCCCGGCCTCCG
CCTCGCTGGTCAAGGCCGTCCGCCGCGCGCGGTCGGCGCAGAAGTCGCGCGGCGACTC
GCACCTCGCCGTCGACCAGCTGCTCATGGGCCTCCTCGAGGACCCGCAGATCTCCGAC
GCGCTCAAGGAGGCCGGCATCTCCGCTGCGCGGGTGAAGGCCGAGGTCGAGAAGCTC
CGGGGTGGCGACAACCGGCGCGTGGAGTCCGCGTCGGGGGACACCAACTTCCAGGCC
CTCAAGACGTACGGCCGCGACCTCGTGGAGGTGGCGGGCAAGCTGGACCCGGTCATC
GGCCGCGACGAGGAGATCCGGCGCGTGGTGCGGATCCTGTCGCGGCGCACAAAGAAC
AACCCCGTCCTCGTCGGCGAGCCCGGCGTGGGCAAGACCGCCGTGGTGGAGGGGCTC
GCGCGGCGCGTCGTGCGCGGCGACGTCCCCAGCAACCTCCTGGACGTGCGCCTGGTCG
CGCTCGACATGGGCGCGCTCGTGGCCGGCGCCAAGTACCGCGGCGAGTTCGAGAGCG
GCTCAAGGCCGTGCTCAAGGAGGTGGAGGAGGCCGAGGGGAAGGTGATACTGTTCAT
CGACGAGATACACCTGGTGCTCGGCGCCGGGCGGACGGAGGGGTCAATGGACGCGGC
CAACCTGTTCAAGCCGATGCTGGCGAGGGGGCGGCTCAGGTGCATTGGCGCGGCGAC
CCTCGAGGAGTACAGGAAGTACG TCGAGA

(A)

Sequence 2 > HSP101 gene
CATGTGGCGGTGTTCAACACTCTGCTCCAGGTCCTGGACGATGGGCGGTTGAC
CGACGGGCAAGGCAGGACGGTTGATTTTAGGAACACGGTGATCATCATGACCT
CAAACCTTGGCGCGGAGCACCTCCTCGCCGGGATGGTGGGCATTTCGATGAAG
GTCGCTCGTGATCTGGTCATGCAGGAGGTGAGGAGGCATTTCCGCCCGGAGCT
GCTGAACCGTCTGGACGAGATCGTCATCTTCGACCCTCTGTCGCATGAGCAGCT
GCGGAGGTCGCTCGGCTTCAGATGAAAGATGTGGCAGTCCGTCTTGCCGAGAG
GGGCGTTGCTCTGGCCGTCACCGACGCCGCCCTGGACGTCATCCTGTCACTGTC
TTACGATGCGGTCTATGGCGCCGGGCCAATCCGGAGATGGATCGAGAAGAGG
ATAGTGACGGAGCTCTCCAAGATGTTGATCCGCGAGGAGATCGACGAGAACTC
CACGGTGTACATCGACGCTGCGCCCAGCAAGGACGAGCTGACCTATGGCGTCG
ACAAGCACGGAGGGCTGGTGAACGCGCGCACGGGCCACAAGTCCGACATCCT
GATCCAGGTTCCTAGCGGAGCTGTTGGGGGCGATGCGGCGCACGCCGTGAAGA
AGATGTAGATCATGCAGGACAGCGGAGAGGTGGACGACGTGGGGGAAGAGTA
GATGGAAACCGCATCGATTGATCCCTTTCTGACTGTAGTTCCAAGTGCCTGCAC
CAGGCAGTCGTATGAGCTCTGTTTTTGCTTTTGGACCGTGCTGATTTAGGTAGT
TCGGAATCGTATCTGTTGAATTGGGGGTGAAATGATTTGTAGTTGAGGATGG

(B)

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

Sequence 3 > Hsp101gene

GGCGCTCGCGGCGGCGCACGAGATGGCGTCCGAGGCCGGCCACGCGCAGCTC
ACGCCGCTGCACCTCGCCGCGGCGCTCGCGGCGGACAGGTCGGGCATCCTCCG
CCAGGCCATCGCCCACGCGTCCGGCGGCAACGACGCCGCGGCCGAGTCGTTCG
AGCGCGTCGCGTCCGCCGCGCTCAAGCGGCTGCCCTCGCAGTCCCCGCCGCCC
GACACCGTCCCGGCCTCCACCTCGCTGGTCAAGGCCGTCCGCCGCGCGCAGTC
GGCGCAGAAGTCGCGCGGCGACTCGCACCTCGCCGTCGACCAGCTGCTCATGG
GCCTCCTCGAGGACCCGCAGATCTCCGACGCGCTCAAGGAGGCCGGCATCTCC
GCTGCGCGGGTGAAGGCCGAGGTCGAGAAGCTCCGGGGAGGCGACAACCGGC
GCGTGGAGTCCGCGTCGGGGGACACCAACTTCCAGGCCCTCAAGACGTACGGC
CGCGACCTCGTGGAGGTGGCGGGCAAGCTGGACCCGGTCATCGGCCGCGACG
AGGAGATCCGGCGCGTGGTGCGGATCCTGTCGCGGCGCACAAAGAACAACCC
CGTCCTCATCGGCGAGCCCGGCGTGGGCAAGACCGCCGTGGTGGAGGGGCTCG
CGCAGCGCGTCGTGCGCGGCGACGTCCCCAGCAACCTCCTGGACGTGCGCCTG
GTCGCGCTCGACATGGGCGCGCTCGTGGCCGGCGCCAAGTACCGCGGCGAGTT
CGAGGAGCGGCTCAAGCCGTGCTCAAGGAGGTGGAGGAGGCCGAGGGGAAGG
TGATACTGTTCATCGACGAGATACACCTGGTGCTCGGCGCCGGGCGGACGGAG
GGGTCAATGGACGCGGCCAACCTGTTCAAGCCGATGCTGGCGAGGGGGCAGCT
CAGGTGCATTGGCGCGACGACCCTCGAGGAGTACAGGAAGTACGTCGAGA

(A)

Sequence 4 > Hsp101gene

CGCATGTGGCGGTGTTCAACACTCTGCTCCAGGTCCTGGACGATGGGCGGTTG
ACCGACGGGCAAGGCAGGACGGTTGATTTTAGGAACACGGTGATCATCATGAC
CTCAAACCTTGGCGCGGAGCACCTCCTCGCCGGAATGGTGGGCAATTCGATGA
AGGTTGCTCGTGATCTGGTCATGCAGGAGGTGAGGAGGCATTTCCGCCCGGAG
CTGCTGAACCGTCTGGACGAGATCGTCATCTTCGACCCTCTGTCGCATGAGCAG
CTGCGGAAGGTCGCTCGGCTTCAGATGAAAGATGTGGCAGTCCGTCTTGCCGA
GAGGGGCGTTGCTCTGGCCGTCACCGACGCCGCCCTGGACGTCATCCTGTCAC
TGTCTTACGATCCGGTCTATGGCGCCAGGCCAATCCGGAGATGGATCGAGAAG
AGGATAGTGACGGAGCTCTCCAAGATGTTGATCCGCGAGGAGATCGACGAGA
ACTCCACGGTGTACATCGACGCTGCGCCCAGCAAGGACGAGCTGACCTATGGC
GTCGACAAGCACGGAGGGCTGGTGAACGCGCGCACGGGCCACAAGTCCGACA
TCCTGATCCAGGTTCCTAGCGGAGCTGTTGGGGGCGATGCGGCGCACGCCGTG
AAGAAGATGAAGATCATGCAGGACAGCGGAGAGGTGGACGACATGGAGGAA
GAGTAGATGGAAACCGCATCGATTATCCCTTTCTGACTGTAGTTCCAAGTGCCT
GCACCAGGCAGTCGTATGAGCTCTGTTTTTGCTTTTGGACCGTGCTGATTTAGG
TAGTTCGGAATCGTATCTGTTGAATTGGGGGTGAAATGAATTGTAGTTGAGGA
TGG

(B)

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

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 *TaHSP101c*	Gemmiza HSP101	Sids HSP101	AF174433.1 *TaHSP101c*	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

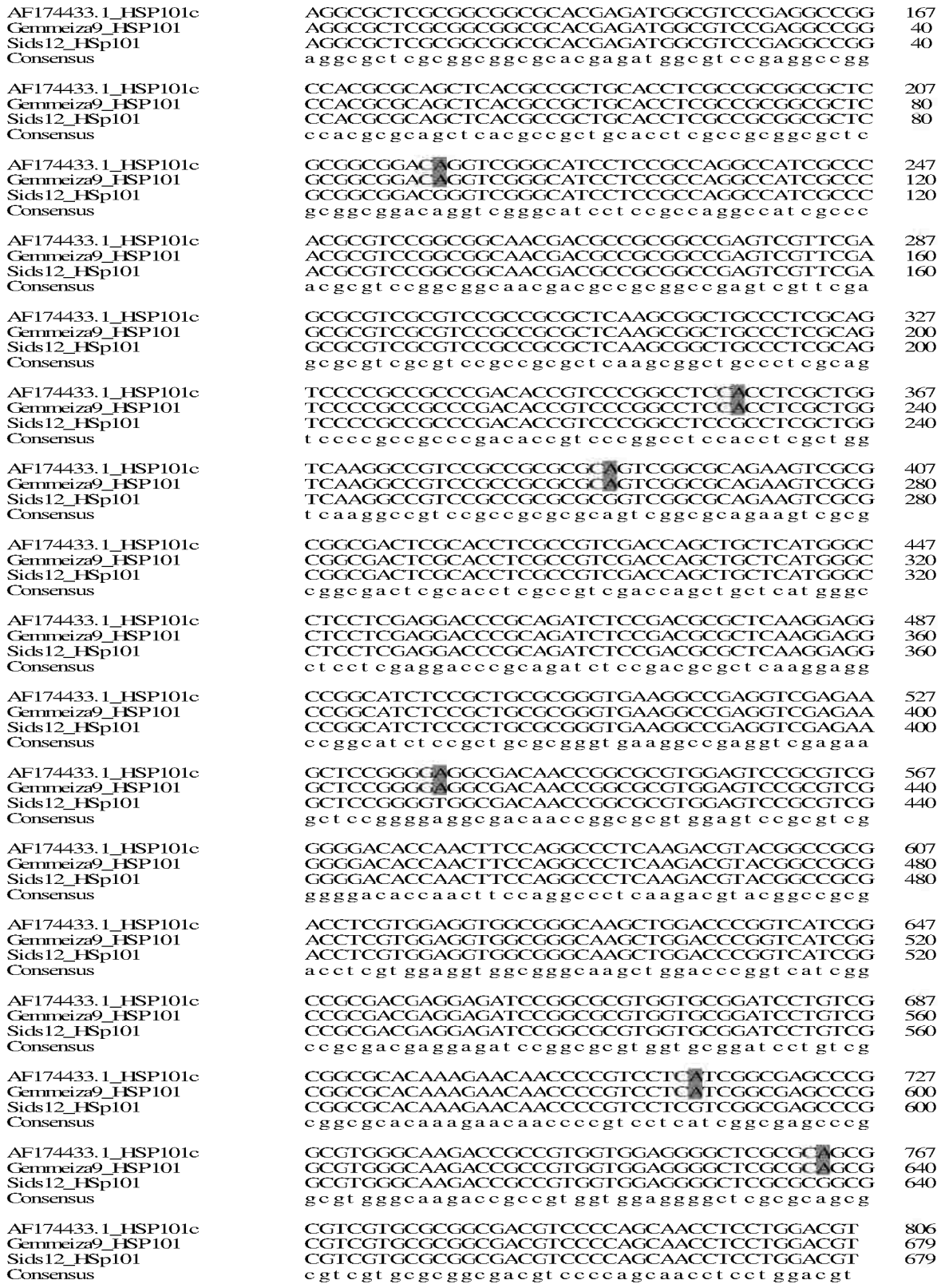
SNPs	Gemmiza9 HSP101	%	Sids 12 HSP101	%
Transition	-	**0%**	**11**	**61%**
Tranversion	-	**0%**	**5**	**28%**
(InDel)	**2**	**100%**	**2**	**11%**
Frequency (bp/SNP)	-		**112**	
Frequency (bp/InDel)	**898**		**898**	

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 varity		
	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 AGGCGCTCCGCGGCGGCGCACGAGATGGCGTCCGAGGCCGG 167
Gemmeiza9_HSP101 AGGCGCTCCGCGGCGGCGCACGAAGATGGCGTCCGAGGCCGG 40
Sids12_HSp101 AGGCGCTCCGCGGCGGCGCACGAGATGGCGTCCGAGGCCGG 40
Consensus a g g c g c t c g c g g c g g c g c a c g a g a t g g c g t c c g a g g c c g g
AF174433.1_HSP101c CCACGCGCAGCTCACGCCGCTGCACCTCGCCGCGGCGCTC 207
Gemmeiza9_HSP101 CCACGCGCAGCTCACGCCGCTGCACCTCGCCGCGGCGCTC 80
Sids12_HSp101 CCACGCGCAGCTCACGCCGCTGCACCTCGCCGCGGCGCTC 80
Consensus c c a c g c g c a g c t c a c g c c g c t g c a c c t c g c c g c g g c g c t c
AF174433.1_HSP101c GCGGCGGACCAGGTCGGGCATCCTCCGCCAGGCCATCGCCC 247
Gemmeiza9_HSP101 GCGGCGGACCAGGTCGGGCATCCTCCGCCAGGCCATCGCCC 120
Sids12_HSp101 GCGGCGGACGGGTCGGGCATCCTCCGCCAGGCCATCGCCC 120
Consensus g c g g c g g a c a g g t c g g g c a t c c t c c g c c a g g c c a t c g c c c
AF174433.1_HSP101c ACGCGTCCGGCGGCAACGACGCCGCGGCCGAGTCGTTCGA 287
Gemmeiza9_HSP101 ACGCGTCCGGCGGCAACGACGCCGCGGCCGAGTCGTTCGA 160
Sids12_HSp101 ACGCGTCCGGCGGCAACGACGCCGCGGCCGAGTCGTTCGA 160
Consensus a c g c g t c c g g c g g c a a c g a c g c c g c g g c c g a g t c g t t c g a
AF174433.1_HSP101c GCGCGTCGCGTCCGCCGCGCTCAAGCGGCTGCCCTCGCAG 327
Gemmeiza9_HSP101 GCGCGTCGCGTCCGCCGCGCTCAAGCGGCTGCCCTCGCAG 200
Sids12_HSp101 GCGCGTCGCGTCCGCCGCGCTCAAGCGGCTGCCCTCGCAG 200
Consensus g c g c g t c g c g t c c g c c g c g c t c a a g c g g c t g c c c t c g c a g
AF174433.1_HSP101c TCCCCGCCGCCCGACACCGTCCCGGCCTCCACCTCGCTGG 367
Gemmeiza9_HSP101 TCCCCGCCGCCCGACACCGTCCCGGCCTCCACCTCGCTGG 240
Sids12_HSp101 TCCCCGCCGCCCGACACCGTCCCGGCCTCCGCCTCGCTGG 240
Consensus t c c c c g c c g c c c g a c a c c g t c c c g g c c t c c a c c t c g c t g g
AF174433.1_HSP101c TCAAGGCCGTCCGCCGCGCGCAGTCGGCGCAGAAGTCGCG 407
Gemmeiza9_HSP101 TCAAGGCCGTCCGCCGCGCGCAGTCGGCGCAGAAGTCGCG 280
Sids12_HSp101 TCAAGGCCGTCCGCCGCGCGCGGTCGGCGCAGAAGTCGCG 280
Consensus t c a a g g c c g t c c g c c g c g c g c a g t c g g c g c a g a a g t c g c g
AF174433.1_HSP101c CGGCGACTCGCACCTCGCCGTCGACCAGCTGCTCATGGGC 447
Gemmeiza9_HSP101 CGGCGACTCGCACCTCGCCGTCGACCAGCTGCTCATGGGC 320
Sids12_HSp101 CGGCGACTCGCACCTCGCCGTCGACCAGCTGCTCATGGGC 320
Consensus c g g c g a c t c g c a c c t c g c c g t c g a c c a g c t g c t c a t g g g c
AF174433.1_HSP101c CTCCTCGAGGACCCGCAGATCTCCGACGCGCTCAAGGAGG 487
Gemmeiza9_HSP101 CTCCTCGAGGACCCGCAGATCTCCGACGCGCTCAAGGAGG 360
Sids12_HSp101 CTCCTCGAGGACCCGCAGATCTCCGACGCGCTCAAGGAGG 360
Consensus c t c c t c g a g g a c c c g c a g a t c t c c g a c g c g c t c a a g g a g g
AF174433.1_HSP101c CCGGCATCTCCGCTGCGCGGGTGAAGGCCGAGGTCGAGAA 527
Gemmeiza9_HSP101 CCGGCATCTCCGCTGCGCGGGTGAAGGCCGAGGTCGAGAA 400
Sids12_HSp101 CCGGCATCTCCGCTGCGCGGGTGAAGGCCGAGGTCGAGAA 400
Consensus c c g g c a t c t c c g c t g c g c g g g t g a a g g c c g a g g t c g a g a a
AF174433.1_HSP101c GCTCCGGGCAGGCCGACAACCGGCGCGTGGAGTCCGCGTCG 567
Gemmeiza9_HSP101 GCTCCGGGCAGGCCGACAACCGGCGCGTGGAGTCCGCGTCG 440
Sids12_HSp101 GCTCCGGGGTGGCCGACAACCGGCGCGTGGAGTCCGCGTCG 440
Consensus g c t c c g g g g g a g g c g a c a a c c g g c g c g t g g a g t c c g c g t c g
AF174433.1_HSP101c GGGGACACCAACTTCCAGGCCCTCAAGACGTACGGCCGCG 607
Gemmeiza9_HSP101 GGGGACACCAACTTCCAGGCCCTCAAGACGTACGGCCGCG 480
Sids12_HSp101 GGGGACACCAACTTCCAGGCCCTCAAGACGTACGGCCGCG 480
Consensus g g g g a c a c c a a c t t c c a g g c c c t c a a g a c g t a c g g c c g c g
AF174433.1_HSP101c ACCTCCGTGGAGGTGGCCGGGCAAGCTGGACCCCGGTCATCGG 647
Gemmeiza9_HSP101 ACCTCCGTGGAGGTGGCCGGGCAAGCTGGACCCCGGTCATCGG 520
Sids12_HSp101 ACCTCCGTGGAGGTGGCCGGGCAAGCTGGACCCCGGTCATCGG 520
Consensus a c c t c g t g g a g g t g g c g g g c a a g c t g g a c c c g g t c a t c g g
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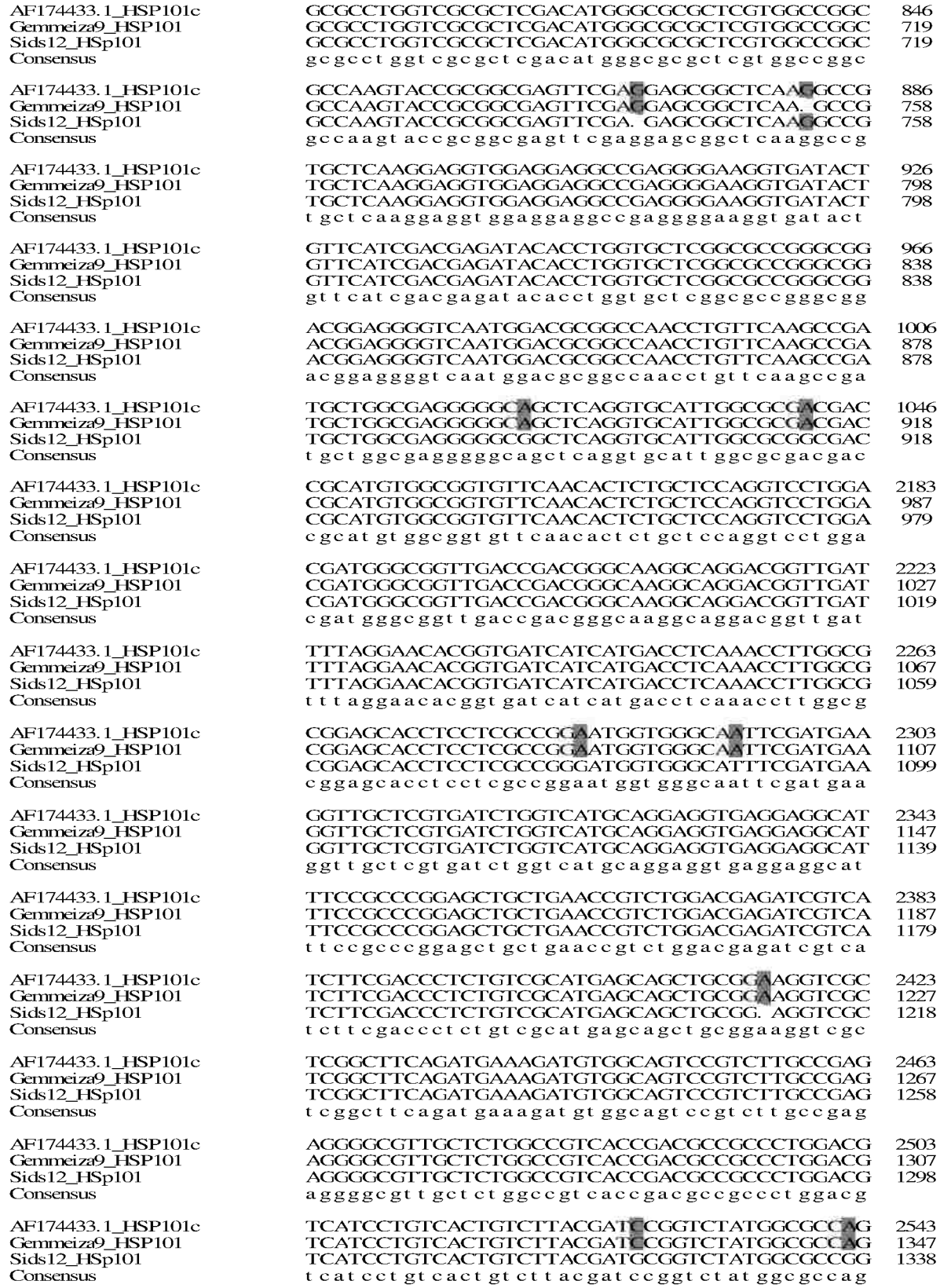
Gemmeiza9_HSP101 CCGCGACGAGGAGATCCGGCGCGTGGTGCGGATCCTGTCG 560
Sids12_HSp101 CCGCGACGAGGAGATCCGGCGCGTGGTGCGGATCCTGTCG 560
Consensus c c g c g a c g a g g a g a t c c g g c g c g t g g t g c g g a t c c t g t c g
AF174433.1_HSP101c CGGCGCACAAAGAACAACCCCCGTCCTCCATCGGCGAGCCCCG 727
Gemmeiza9_HSP101 CGGCGCACAAAGAACAACCCCCGTCCTCCATCGGCGAGCCCCG 600
Sids12_HSp101 CGGCGCACAAAGAACAACCCCCGTCCTCCGTCGGCGAGCCCCG 600
Consensus c g g c g c a c a a a g a a c a a c c c c c g t c c t c a t c g g c g a g c c c c g
AF174433.1_HSP101c GCGTGGGCAAGACCGCCGTGGTGGAGGGGCTCCGCCAGCG 767
Gemmeiza9_HSP101 GCGTGGGCAAGACCGCCGTGGTGGAGGGGCTCCGCCGCGGCG 640
Sids12_HSp101 GCGTGGGCAAGACCGCCGTGGTGGAGGGGCTCCGCCGCGGCG 640
Consensus g c g t g g g c a a g a c c g c c g t g g t g g a g g g g c t c g c g c a g c g
AF174433.1_HSP101c CGTCGTGCGCGGCGACGTCCCCAGCAACCTCCTGGACGT 806
Gemmeiza9_HSP101 CGTCGTGCGCGGCGACGTCCCCAGCAACCTCCTGGACGT 679
Sids12_HSp101 CGTCGTGCGCGGCGACGTCCCCAGCAACCTCCTGGACGT 679
Consensus c g t c g t g c g c g g c g a c g t c c c c a g c a a c c t c c t g g a c g t

(A)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Sids12_HSp101 GCGCCTGGTCGCGCTCGACATGGGCGCGCTCGTGGCCGGC 719
Consensus gcgcct ggt cgcgct cgacat gggcgcgct cgt ggccggc
AF174433.1_HSP101c GCCAAGTACCGCGGCGAGTTCGAGGAGCGGCTCAAGGCCG 886
Gemmeiza9_HSP101 GCCAAGTACCGCGGCGAGTTCGAGGAGCGGCTCAA. GCCG 758
Sids12_HSp101 GCCAAGTACCGCGGCGAGTTCGA. GAGCGGCTCAAGGCCG 758
Consensus gccaagt accgcggcgagt t cgaggagcggct caaggccg
AF174433.1_HSP101c TGCTCAAGGAGGTGGAGGAGGCCGAGGGGAAGGTGATACT 926
Gemmeiza9_HSP101 TGCTCAAGGAGGTGGAGGAGGCCGAGGGGAAGGTGATACT 798
Sids12_HSp101 TGCTCAAGGAGGTGGAGGAGGCCGAGGGGAAGGTGATACT 798
Consensus t gct caaggaggt ggaggaggccgaggggaaggt gat act
AF174433.1_HSP101c GTTCATCGACGAGATACACCTGGTGCTCGGCGCCGGGCGG 966
Gemmeiza9_HSP101 GTTCATCGACGAGATACACCTGGTGCTCGGCGCCGGGCGG 838
Sids12_HSp101 GTTCATCGACGAGATACACCTGGTGCTCGGCGCCGGGCGG 838
Consensus gt t cat cgacgagat acacct ggt gct cggcgccgggcgg
AF174433.1_HSP101c ACGGAGGGGTCAATGGACGCGGCCAACCTGTTCAAGCCGA 1006
Gemmeiza9_HSP101 ACGGAGGGGTCAATGGACGCGGCCAACCTGTTCAAGCCGA 878
Sids12_HSp101 ACGGAGGGGTCAATGGACGCGGCCAACCTGTTCAAGCCGA 878
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Gemmeiza9_HSP101 TGCTGGCGAGGGGGGCAGCTCAGGTGCATTGGCGCGACGAC 918
Sids12_HSp101 TGCTGGCGAGGGGGGCGGCTCAGGTGCATTGGCGCGGCGAC 918
Consensus t gct ggcgaggggggcagct caggt gcat t ggcgcgacgac
AF174433.1_HSP101c CGCATGTGGCGGTGTTCAACACTCTGCTCCAGGTCCTGGA 2183
Gemmeiza9_HSP101 CGCATGTGGCGGTGTTCAACACTCTGCTCCAGGTCCTGGA 987
Sids12_HSp101 CGCATGTGGCGGTGTTCAACACTCTGCTCCAGGTCCTGGA 979
Consensus cgcat gt ggcggt gt t caacact ct gct ccaggt cct gga
AF174433.1_HSP101c CGATGGGCGGTTGACCGACGGGCAAGGCAGGACGGTTGAT 2223
Gemmeiza9_HSP101 CGATGGGCGGTTGACCGACGGGCAAGGCAGGACGGTTGAT 1027
Sids12_HSp101 CGATGGGCGGTTGACCGACGGGCAAGGCAGGACGGTTGAT 1019
Consensus cgat gggcggt t gaccgacgggcaaggcaggacggt t gat
AF174433.1_HSP101c TTTAGGAACACGGTGATCATCATGACCTCAAACCTTGGCG 2263
Gemmeiza9_HSP101 TTTAGGAACACGGTGATCATCATGACCTCAAACCTTGGCG 1067
Sids12_HSp101 TTTAGGAACACGGTGATCATCATGACCTCAAACCTTGGCG 1059
Consensus t t t aggaacacggt gat cat cat gacct caaaccttggcg
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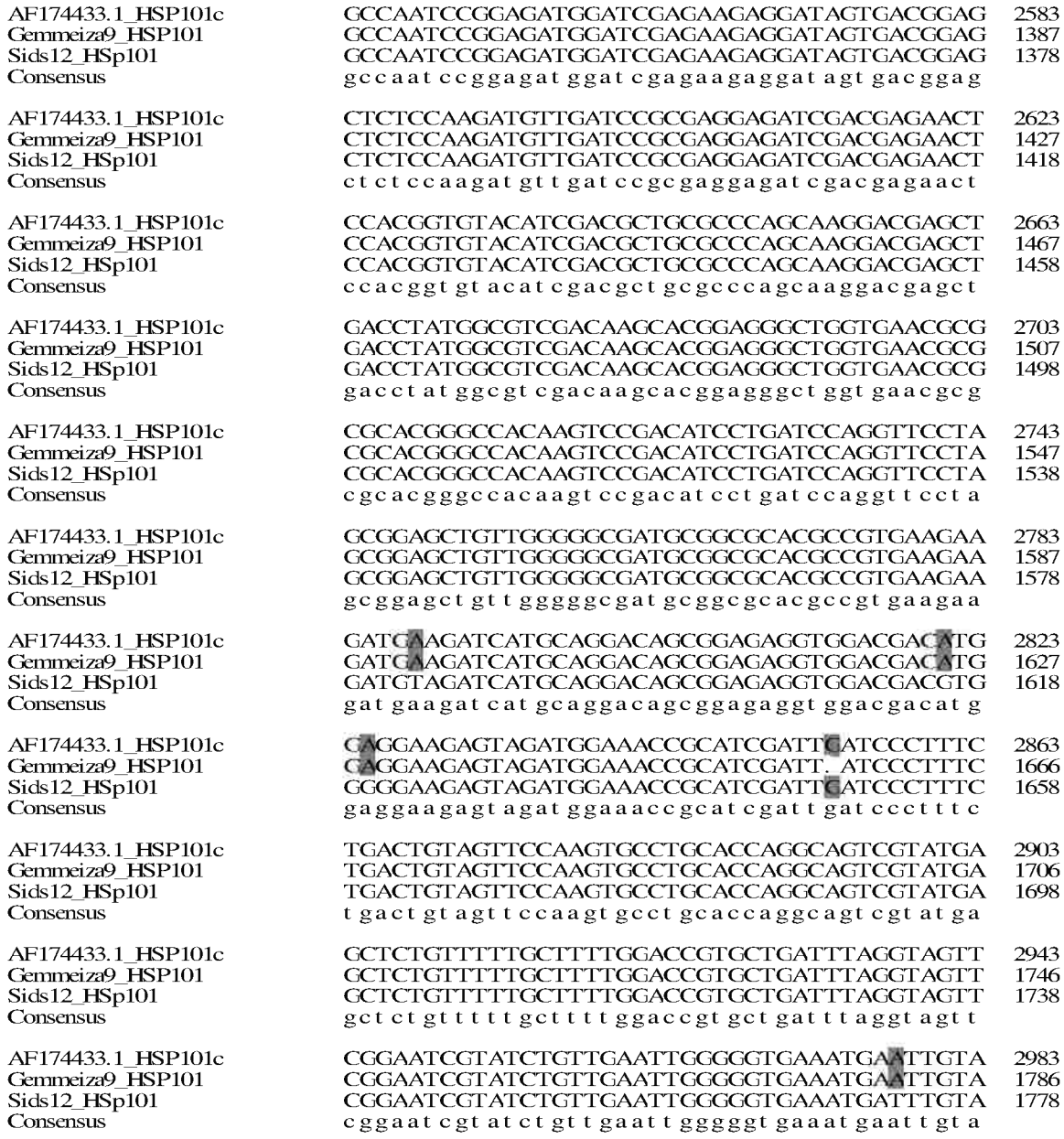
Gemmeiza9_HSP101 CGGAGCACCTCCTCCGCCGGAATGGTGGGCAATTCGATGAA 1107
Sids12_HSp101 CGGAGCACCTCCTCCGCCGGGATGGTGGGCATTTCGATGAA 1099
Consensus cggagcacct cct cgccggaat ggt gggcaat t cgat gaa
AF174433.1_HSP101c GGTTGCTCGTGATCTGGTCATGCAGGAGGTGAGGAGGCAT 2343
Gemmeiza9_HSP101 GGTTGCTCGTGATCTGGTCATGCAGGAGGTGAGGAGGCAT 1147
Sids12_HSp101 GGTTGCTCGTGATCTGGTCATGCAGGAGGTGAGGAGGCAT 1139
Consensus ggt t gct cgt gat ct ggt cat gcaggaggt gaggaggcat
AF174433.1_HSP101c TTCCGCCCCGGAGCTGCTGAACCGTCTGGACGAGATCGTCA 2383
Gemmeiza9_HSP101 TTCCGCCCCGGAGCTGCTGAACCGTCTGGACGAGATCGTCA 1187
Sids12_HSp101 TTCCGCCCCGGAGCTGCTGAACCGTCTGGACGAGATCGTCA 1179
Consensus t t ccgcccggagct gct gaaccgt ct ggacgagat cgt ca
AF174433.1_HSP101c TCTTCGACCCTCTGTCGCATGAGCAGCTGCGCGAAGGTCGC 2423
Gemmeiza9_HSP101 TCTTCGACCCTCTGTCGCATGAGCAGCTGCGCGAAGGTCGC 1227
Sids12_HSp101 TCTTCGACCCTCTGTCGCATGAGCAGCTGCGG. AGGTCGC 1218
Consensus t ct t cgaccct ct gt cgcat gagcagct gcggaaggt cgc
AF174433.1_HSP101c TCGGCTTCAGATGAAAGATGTGGCAGTCCGTCTTGCCGAG 2463
Gemmeiza9_HSP101 TCGGCTTCAGATGAAAGATGTGGCAGTCCGTCTTGCCGAG 1267
Sids12_HSp101 TCGGCTTCAGATGAAAGATGTGGCAGTCCGTCTTGCCGAG 1258
Consensus t cggct t cagat gaaagat gt ggcagt ccgt ct t gccgag
AF174433.1_HSP101c AGGGGCGTTGCTCTGGCCGTCACCGACGCCGCCCTGGACG 2503
Gemmeiza9_HSP101 AGGGGCGTTGCTCTGGCCGTCACCGACGCCGCCCTGGACG 1307
Sids12_HSp101 AGGGGCGTTGCTCTGGCCGTCACCGACGCCGCCCTGGACG 1298
Consensus aggggcgt t gct ct ggccgt caccgacgccgccct ggacg
AF174433.1_HSP101c TCATCCTGTCACTGTCTTACGATCCGGTCTATGGCGCCAG 2543
Gemmeiza9_HSP101 TCATCCTGTCACTGTCTTACGATCCGGTCTATGGCGCCAG 1347
Sids12_HSp101 TCATCCTGTCACTGTCTTACGATGCGGTCTATGGCGCCGG 1338
Consensus t cat cct gt cact gt ct t acgat ccggt ct at ggcgccag

(B)



(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)

```
201 GGCGCTCGCGGCGGACAAGGTCGGGCATCCTCCGCCAGGCCATCGCCCACG AF174433.1 [1,600]
201 GGCGCTCGCGGCGGACAAGGTCGGGCATCCTCCGCCAGGCCATCGCCCACG Gemmeiza9  [1,473]
201 GGCGCTCGCGGCGGACGGGTCGGGCATCCTCCGCCAGGCCATCGCCCACG Sids12     [1,473]

351 GGCCTCCACCTCGCTGGTCAAGGCCGTCCGCCGCGCGCAGTCGGCGCAGA AF174433.1 [1 600]
351 GGCCTCCACCTCGCTGGTCAAGGCCGTCCGCCGCGCGCAGTCGGCGCAGA Gemmeiza9 [1, 473]
351 GGCCTCCGCCTCGCTGGTCAAGGCCGTCCGCCGCGCGCGGTCGGCGCAGA Sids12    [1, 473]

501 TGCGCGGGTGAAGGCCGAGGTCGAGAAGCTCCGGGCAGGCGACAACCGGC AF174433.1 [1,600]
501 TGCGCGGGTGAAGGCCGAGGTCGAGAAGCTCCGGGCAGGCGACAACCGGC Gemmeiza9 [1, 473]
501 TGCGCGGGTGAAGGCCGAGGTCGAGAAGCTCCGGGGTGGCGACAACCGGC Sids12    [1, 473]
```

(B)

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

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

251 AGTACCGCGGCGAGTTCGACGAGCGGCTCAACGCCGTGCTCAAGGAG AF174433.1 [601,1200]
251 AGTACCGCGGCGAGTTCGACGAGCGGCTCAAC-CCGTGCTCAAGGAG Gemmeiza9  [474, 947]
251 AGTACCGCGGCGAGTTCGAC-AGCGGCTCAACGCCGTGCTCAAGGAG Sids12     [474, 941]

401 AGCCGATGCTGGCGAGGGGGCAGCTCAGGTGCATTGGCGCGACGACC AF174433.1 [601,1200]
401 AGCCGATGCTGGCGAGGGGGCAGCTCAGGTGCATTGGCGCGACGACC Gemmeiza9  [474, 947]
401 AGCCGATGCTGGCGAGGGGGCGGCTCAGGTGCATTGGCGCGGCGACC Sids12     [474, 941]
```

(C)

```
151 TCAAACCTTGGCGCGGAGCACCTCCTCGCCGCAATGGTGGGCAATT AF174433.1 [2101,2458]
151 TCAAACCTTGGCGCGGAGCACCTCCTCGCCGCAATGGTGGGCAATT Gemmeiza9  [948, 1262]
151 TCAAACCTTGGCGCGGAGCACCTCCTCGCCGCGATGGTGGGCATTT Sids12     [940, 1253]

301 CATGAGCAGCTGCGGAAGGTCGCTCGGCTTCAGATGAAAGATGTGG AF174433.1 [2101,2458]
301 CATGAGCAGCTGCGGAAGGTCGCTCGGCTTCAGATGAAAGATGTGG Gemmeiza9  [948, 1262]
301 CATGAGCAGCTGCGGA-GGTCGCTCGGCTTCAGATGAAAGATGTGG Sids12     [940, 1253]
```

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 compostion 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 a*t 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 precentag 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.

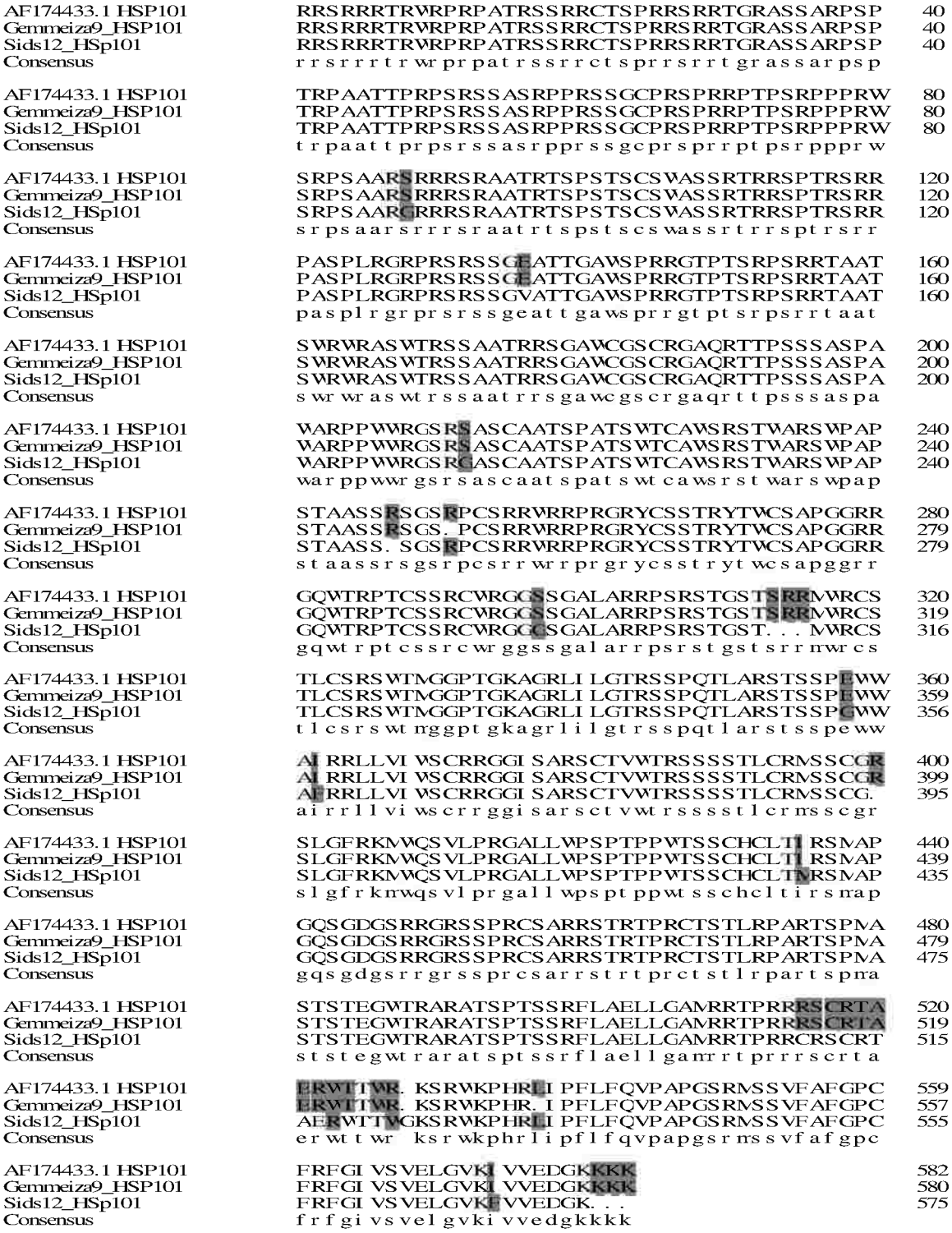


Figure (20): Alignment of the deduced amino acid sequences of TdHSP101c, Gemmeiza9 HSP101 and Sids12 HSP101 obtained with DNAMAN® software (Lyon BioSoft, Quebec, Canada). Gaps in the sequences are indicated by dots ‘‘.’’ Indicate conserved consensus sequences at last sequence. Indicate variance amino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

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

Amino acid position change	***AF174433.1(HSP101c)***	***Sids12_HSp101***	***Gemmeiza9_HSP101***
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**

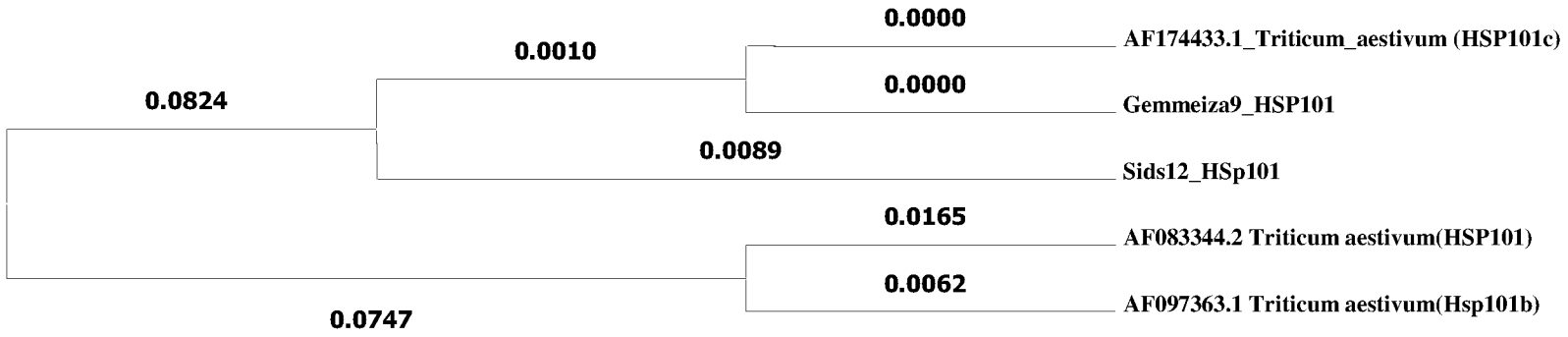


Figure (21): Dendrogram of genetic distance based on the bread wheat Hsp101 gene single nucleotide polymorphism markers.constructed using MEGA6.06

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