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The Effect of Heat Stress on Morpho Physiological Traits of *Triticum Aestivum* L. Genotypes

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Abstract: Heat stress remains a major environmental factor which decreases the yield and productivity of most cereals growing worldwide. The research work was performed to analyze the influence of heat stress on the performance of morpho-physiological characteristics of *Triticum aestivum* genotypes at NARC Islamabad, Pakistan during 2014 - 2015. Various traits of wheat were evaluated by using complete randomize design with triplicates. Analysis of variance reveals adverse influence of heat stress on observed traits of selected genotypes. The result indicates that extreme temperature causes reduction to grain yield, yield per plant. All genotypes responded different against heat stress as compared to optimum temperature. Among all varieties, genotypes 1067, 1123, 1124, 1137, 1154, 1159 and 1163 confirmed most tolerable to heat stress regarding seeds yield per plant, proline content, membrane stability index and total chlorophyll contents. It is suggested that heat stress tolerant varieties should be used in plant breeding programs in the development of different wheat varieties having heat stress tolerance at different stages of plant growth and further research studies should be investigated for the progress of potential heat acceptable genotypes in high temperature regimes.

Keywords: Heat Stress, Genotypes, Wheat, Environmental Factor

INTRODUCTION

High temperature remains a huge problem for edible crops world-wide with unexpected spatial and temporal variations causing reduced crops' yield and productivity (Parent et al., 2010). It is known that an increase in temperature of 1 °C in different crops reduces yield 3–10 percent (You et al., 2009). Wheat is an important crop of Pakistan where the estimated per capita consumption is about 124 kg per year. In order to meet the demand for food in Pakistan, an increase in wheat crop production of at least four percent is required to sustain population growth. In Pakistan, wheat yield is sensitive to heat stress in flowering and seed ripening stages. During these periods, heat stress delays the growth, causes premature ripening, decrease the number of grain, decreases grain weight and ultimately results in decrease in total grains yield and quality deterioration (Khan et al., 2007, Wahid et al., 2007, Din et al. 2010). It is estimated that one-degree increase in temperature above optimum temperature for wheat growth could minimize the wheat yields about 3-10 percent (You et al., 2009). Wheat production is delicate to heat stress as high temperature declines wheat production by 20-30 percent in different developing countries. Global warming adversely affects wheat grain yield, which increases food uncertainty and shortage (Ortiz et al., 2008). Therefore, there is an urgent need to develop different wheat cultivars which can be able to tolerate heat stress at different vegetative and

reproductive stages of plant growth seasons. An increase in proline concentration also leads to tolerate the crops against heat stress (Ahmed & Hasan, 2011). Accumulation of proline had shown to occur at heat stress in *Arabidopsis thaliana* (Wei-Tao et al., 2011), *Helianthus annuus* (Ronde et al., 2001) and *Triticum aestivum* (Hasan et al., 2007). Chlorophyll content is a required trait in genotypes exposed to heat stress because it prevents photo inhibition; hence, decreasing carbohydrate losses for grain development (Ananthi et al., 2013). Madhan et al., (2000) confirms that stability of chlorophyll under stress is very significant and considered as chlorophyll stability index. High CSI helps the crops to survive in stress situations as it led to high photosynthesis. Mohammadi et al., (2008) reported that wheat cultivar with high chlorophyll content has more yield and considered as valuable character for selection of improved wheat genotypes. Membrane thermal stability in case of heat stress typically measured as ion leakage in cell was used for screening *Triticum aestivum* germplasm for thermal tolerance (Yildirim et al., 2009). Blum et al., (2001) showed a higher yield in spring wheat lines having greater membrane-thermo stability in flag leaves at anthesis. Irfan et al., (2017) documented that salt stress can be overcome by the influence of brassinosteroid. The study was conducted to investigate the physiological traits for heat stress tolerance at various vegetative and reproductive stages in *Triticum aestivum* and reliable strategies for heat stress tolerance that can be utilized in *Triticum aestivum* breeding programs in Pakistan and other countries.

Materials and Method:

The plant materials which were used in research work was a set of 10 selected International Bread Wheat Screening Nursery (45-IBWSN) genotypes of CIMMYT 1038, 1067, 1114, 1121, 1123, 1124, 1137, 1154, 1159, 1163. The Wheat genotypes were collected from wheat wide crosses and Cytogenetic Lab National Agriculture Research Centre, (NARC) Islamabad. The experimental design was made in green house in National Agriculture Research Centre Islamabad, Pakistan during the winter season of 2014-2015. Complete randomized design was applied with three replications. The seed of selected ten genotypes were sown in pots filled with sandy soil. The water was given at normal rate to the control pots and heat stress was given to the treated pots. In heat stress treatments the plants when reached at post anthesis stage shifted to the green house for more heat treatments. The different heat stress treatments were provided for consecutive seven days and daily for five hours' treatment was provided, where the glass house internal temperature was minimum 38°C and maximum 40°C and all the treatments were given water properly during stress period. At maturity stages, different morpho-physiological traits viz. seeds yield per plant, 1000 grain weight, number of grains per spike, chlorophyll contents, membrane stability index and total proline contents were studied from different selected (IBWSN) *Triticum aestivum* genotypes at the appropriate stage to examine variation in qualitative and quantitative traits.

Grain yield per plant: All spikes of each studied plant were rubbed by hand and weighed. Average grain yield of all genotypes in every replicate per plant was valued.

1000 grain weight: The grains from nine chosen particular spikes of plants in all replicates of each genotype were bulked separately. 1000-grains were count up at chance from each bulk and weighed in grams.

Number of grains per spike: For genotype the spikes of the mother shoot were threshed and numbers of grains per spikes were counted each replication of every genotypes.

Total Proline contents of leaves:

After daily five hours' heat stress treatments for seven consecutive days' flag leaf of control trial and heat stressed plants was sampled for analysis of total proline contents. Total proline contents were

analyzed by using the method of (Bates et al., 1973). Fresh tissues of plant samples were extracted with three percent of aqueous five - sulfosalicylic acid and filtrate was reacted with acetic acid and ninhydrin solution at 100 °C for one hour. The mixture was extracted with toluene and the absorbance of the chromophore contain toluene were studied at 520 nm.

Total chlorophyll contents of leaves:

One ml crude leaf preparation was mixed with ten ml of eighty percent ethanol and permitted to stand in the dark at room temperature for ten minutes and then centrifuged at two thousand revolutions per minute for five minutes to clear the suspension. For the determination of chlorophyll supernatant which contained soluble pigment was used. Absorbance of solution was read at 663 nm for the measurement of chlorophyll b and at 645 nm for chlorophyll a on photo spectrophotometer against 80% ethanol blank by following the equation given by (Arnon, 1949) total chlorophyll was determined

$$\text{Total chlorophyll (mg/g)} = 20.2 \cdot a_{645\text{nm}} + (8.02 \cdot b_{663})$$

Leaf membrane stability index:

The leaf membrane stability index was evaluated according to method of (Premachandra et al., 1990) which was modified by (Sairam, 1994). It was determined in test tubes containing distilled water in two sets of leaves pieces (0.2 grams) were taken at 40°C for thirty minutes. Test tubes of one set were kept in water and electrical conductivity of water containing samples were measured by using a conductivity meter at 100°C in the boiling water for fifteen minutes. Test tubes in the second set were incubated and their electrical conductivity was measured by using the formula as given below and membrane stability index was calculated and expressed on percentage basis.

$$\text{MSI} = [1 - C1/C2] \times 100$$

The recorded data were computed and analyzed by using different computer softwares including STATISTIC 8.1 and SPSS 20.

Results:

Effect of heat stress on seed yield per plant and 1000 seed weight:

The heat treatment significantly decreased the seed yield per plant in all the tested *Triticum aestivum* genotypes at anthesis stage and there is a significant variation among the genotypes at anthesis stage (table. 1) and the anthesis stage is more sensitive to heat stress.

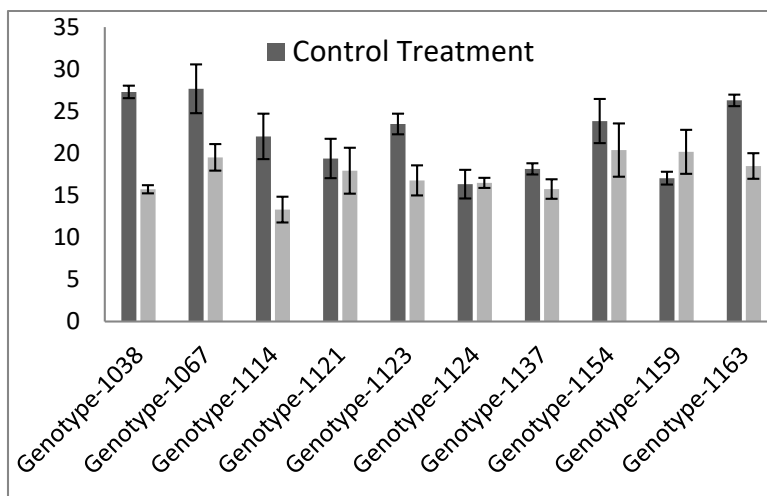


Figure 1: Yield per plant in normal and heat stress condition

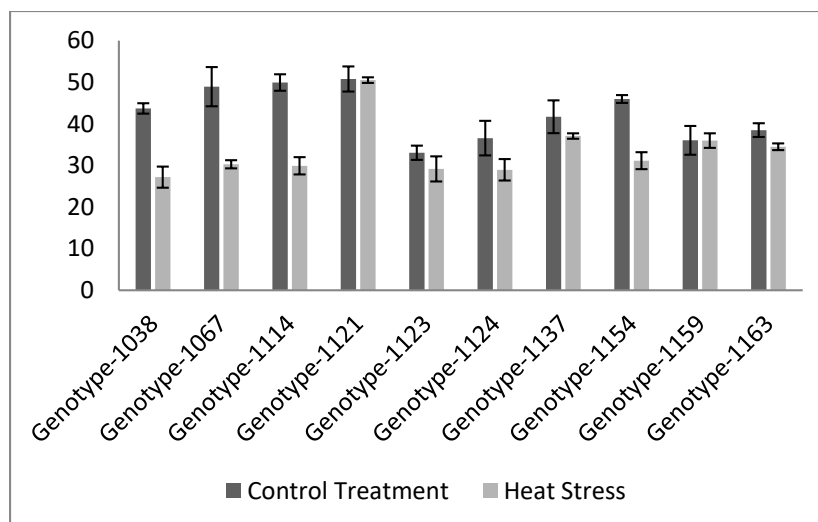


Figure 2: 1000-grains weight in normal and heat stress condition

The genotype-1124 had decreased yield (16.337g) at normal temperature while the same genotype had (16.487g) yield under raised temperature. In normal condition increased yield (27.677 gm 27.303gm and 26.303gm) were recorded in genotypes 1067, 1038 and 1163 while under raised temperature these genotypes had (19.517 gm, 15.733 gm and 18.490 gm) yield per plant respectively (table 1).

1000 seed weight decreased as a result of heat treatment for all genotypes as compared to normal environment condition. Most of genotypes had significantly influences of treatments were recorded on 1000- grain weight in all studied genotypes minimum 1000-grains weight (33.383 gm and 29.15 gm) was noted in genotype -1123 for normal and raised heat treatments; however, genotype -1121 had more 1000 - seeds weight (51.033 gm and 50.50 gm) for normal and heat stress treatments (table 1).

Table 1: The effect of heat stress applied at anthesis stage on yield per plant and 1000 seeds weight of Triticum aestivum genotypes

| S. No | Genotypes | Yield per plant (g) | | 1000-grains weight (g) | |
|-------|-----------|---------------------|-----------------------|------------------------|-----------------------|
| | | Normal condition | Heat stress condition | Normal condition | Heat stress condition |
| 1 | 1038 | 27.303AB | 15.733AB | 44.250ABCD | 27.187E |
| 2 | 1067 | 27.677A | 19.517A | 49.450AB | 30.257DE |
| 3 | 1114 | 22.017BCDE | 13.310B | 50.283AB | 29.903DE |
| 4 | 1121 | 19.390CDEF | 17.937AB | 51.033A | 50.500A |
| 5 | 1123 | 23.490ABCD | 16.777AB | 33.383E | 29.150DE |
| 6 | 1124 | 16.337F | 16.487AB | 36.553DE | 28.813E |
| 7 | 1137 | 18.150DEF | 15.753AB | 41.880BCDE | 37.073B |
| 8 | 1154 | 23.837ABC | 20.387A | 46.063ABC | 31.137CDE |
| 9 | 1159 | 17.043EF | 20.183A | 36.377DE | 35.963BC |
| 10 | 1163 | 26.303AB | 18.490AB | 38.717CDE | 34.467BCD |
| Lsd | | 7.521 | 5.648 | 9.053 | 5.622 |

The values given in column having same letter are not significantly different $p > 0.05$, Duncan's multiple range test

Impact of heat stress on proline, chlorophyll contents and membrane stability index:

Heat stress applied at anthesis significantly increased proline contents in leaves of all Triticum aestivum genotypes as compared to their respective control values. It is recorded that proline

concentration remains higher at heat stress over normal condition. The minimum proline contents were (54.667 and 186.67mg/g) noted in genotype – 1038 at control and heat stress; however, maximum proline contents were (77.0 mg/g) noted in genotypes 1124 and 1163 but these genotypes had 265.33 and 252.0 mg/g proline in heat stress condition.

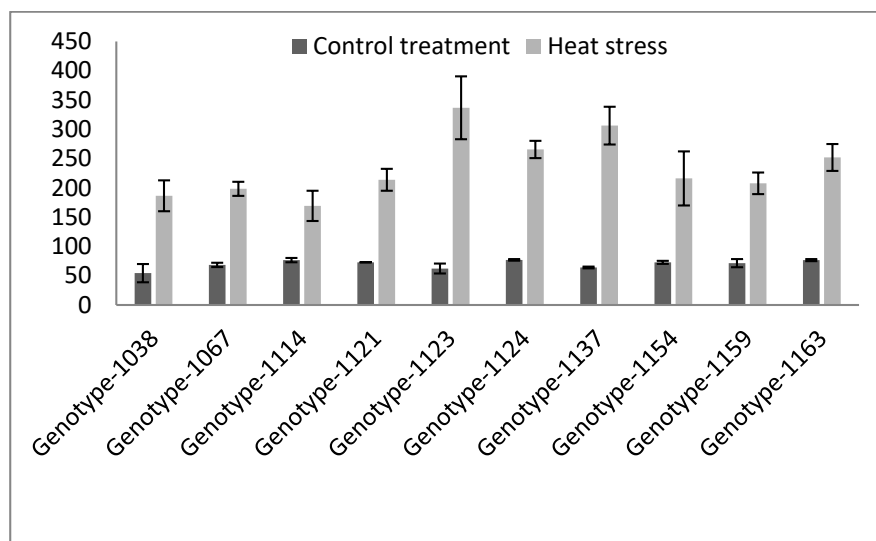


Figure 3. The effect of heat stress on leaf proline concentration (mg/g) at anthesis stage of *Triticum aestivum* genotypes.

Vertical bars indicate standard error. All means are significantly different at $p < 0.05$.

It is studied that membrane stability index were decreased in heat stress condition than normal condition. The minimum membrane stability index (60.667%) were noted in genotype-1038 under both treatments whereas maximum membrane stability index (89.667and 78.0%) was confirmed in genotypes of 1159 and 1137 under normal condition and these genotypes had 78.0 and 66.0% membrane stability index in heat stress treatment (Fig 3 & Table 2).

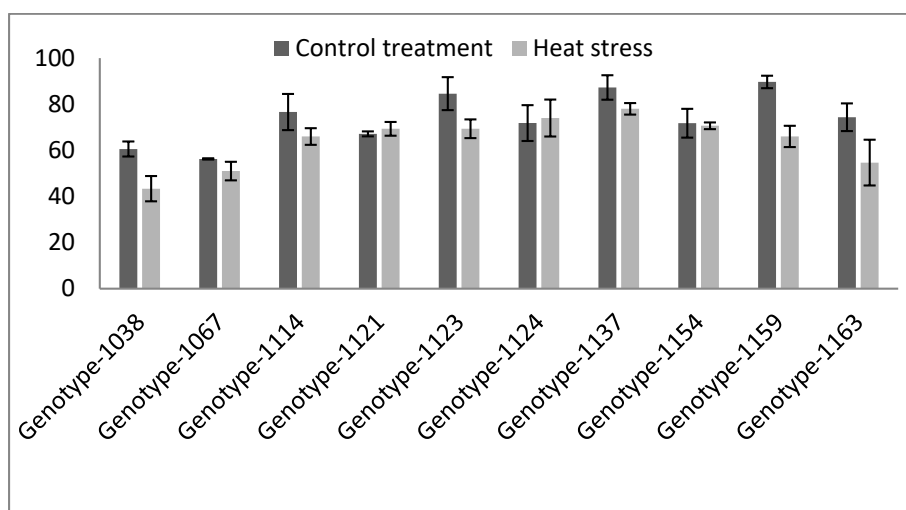


Figure 4. The effect of heat stress on the membrane stability index (%) at anthesis stage of *Triticum aestivum* genotypes.

Vertical bars indicate standard error. All means are significantly different at $p < 0.05$.

Table 2: The effect of heat stress applied at anthesis stage on proline content and membrane stability index of *Triticum aestivum* genotypes

| S. No | Genotypes | Proline contents (mg/g) | | MSI (%) | |
|-------|-----------|-------------------------|-----------------------|------------------|-----------------------|
| | | Normal condition | Heat stress condition | Normal condition | Heat stress condition |
| 1 | 1038 | 54.667B | 186.67CD | 60.667CD | 60.667D |
| 2 | 1067 | 68.667AB | 198.67BCD | 56.333D | 51.000CD |
| 3 | 1114 | 76.667A | 169.33D | 76.333ABC | 66.000ABC |
| 4 | 1121 | 73.000AB | 214.00BCD | 67.333CD | 69.000AB |
| 5 | 1123 | 62.333AB | 336.67A | 84.333AB | 69.000AB |
| 6 | 1124 | 77.000A | 265.33ABC | 72.000BCD | 74.000A |
| 7 | 1137 | 64.000AB | 289.33AB | 87.333AB | 78.000A |
| 8 | 1154 | 71.333AB | 216.00BCD | 72.000BCD | 70.667A |
| 9 | 1159 | 71.667AB | 207.67BCD | 89.667A | 66.000ABC |
| 10 | 1163 | 77.000A | 252.00ABCD | 74.333ABC | 54.667BCD |
| CV | | 18.681 | 91.792 | 16.374 | 15.542 |

Under heat stress decrease in total chlorophyll contents was recorded at anthesis stage as compared to the control treatment. Table 3 shows mean values of chlorophyll contents in evaluated genotypes under stress condition and indicates significant variations in few genotypes, while non-significant differences in most of germ plasm. The mean values show that decrease in chlorophyll (a) among genotypes ranges from 1.213 to 2.87 mg/g in normal condition while it was from 0.456 to 0.66 mg/g under heat stress (Fig 5). At normal temperature, chlorophyll (b) ranged from 0.436 to 1.233 mg/g and at heat stress it was from 0.203 to 0.263 mg/g (Table 6). Total chlorophyll contents ranged from 1.793 to 3.30 mg/g at normal temperature and 0.663 to 1.150 mg/g at heat stress (Fig 6). The results show that genotype-1038 had minimum chlorophyll-a (1.213 mg/g) under normal condition and (0.456 mg/g) at heat stress.

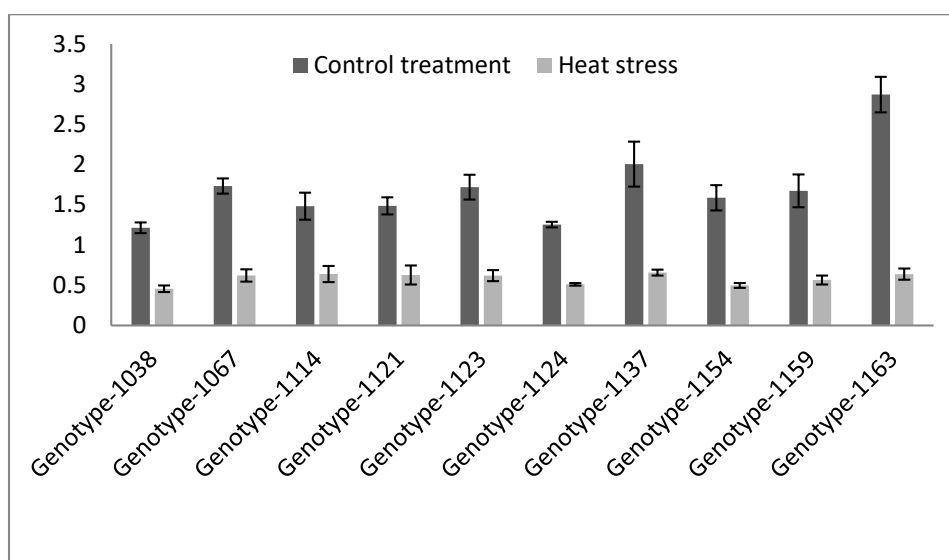


Figure 5: Concentration of chlorophyll-a in control and heat stress conditions

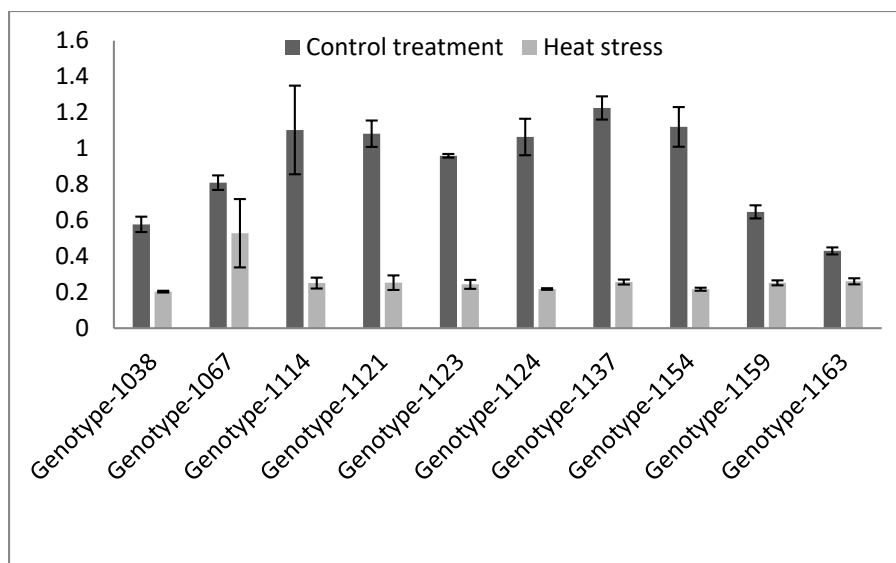


Figure 6: Concentration of chlorophyll-b in control and heat stress conditions

Table 3: The effect of heat stress applied at anthesis stage on chlorophyll contents of *Triticum aestivum* genotypes.

Values given in column having same letter are significantly different $p > 0.05$, Duncan’s multiple range test.

| S. No | Genotypes | Chlorophyll-a (mg/g) | | Chlorophyll-b (mg/g) | |
|-------|-----------|----------------------|-----------------------|----------------------|-----------------------|
| | | Normal condition | Heat stress condition | Normal condition | Heat stress condition |
| 1 | 1038 | 1.213D | 0.456B | 0.576CD | 0.203B |
| 2 | 1067 | 1.733BC | 0.620AB | 0.810BC | 0.530A |
| 3 | 1114 | 1.486CD | 0.640AB | 1.103AB | 0.250B |
| 4 | 1121 | 1.483CD | 0.626AB | 1.083AB | 0.253B |
| 5 | 1123 | 1.716BC | 0.620AB | 0.960AB | 0.243B |
| 6 | 1124 | 1.253CD | 0.510AB | 1.063AB | 0.216B |
| 7 | 1137 | 2.006B | 0.660A | 1.233A | 0.256B |
| 8 | 1154 | 1.586BCD | 0.500AB | 1.183A | 0.216B |
| 9 | 1159 | 1.673BCD | 0.560AB | 0.646CD | 0.253B |
| 10 | 1163 | 2.870A | 0.636AB | 0.436D | 0.263B |
| CV | | 0.483 | 0.201 | 0.297 | 0.186 |

Discussion:

Heat stress provided after anthesis resulted changes in physiological parameters viz. total proline contents, total soluble proteins, membrane stability index and yield parameters. Susceptibility to heat stress varies with the stage of plant development (Wahid et al., 2007). The result concluded that ten *Triticum aestivum* genotypes were exposed to heat stress at anthesis stage causes morphological and physiological changes and affect yield and yield components. Resistance of germplasm to heat stress might be diverging according to growth stage of plants. Heat stress convinced alterations in wheat with a change in physiological progressions and from the fluctuating pattern of growth. Outcomes from present findings showed that all the genotypes were adversely affected under heat stress regarding to 1000 seeds weight. Three genotypes including 1121, 1137 and 1159 showed increase of 1000 seeds weight; however, genotype 1038 had decreased weight under stress condition. Though all the selected

wheat lines revealed decrease in 1000 seeds weight due to heat stress, the seeds' development period loss in seeds weight is due to the injury caused by high temperature. Moraes et al., (2008), Calderini et al., (1997) and Wardlaw, (2002) confirm that decrease in grain weight occur at pre and post anthesis stages. In the current research, a significant increase was noted for grain yield per plant in genotypes including 1067, 1154 and 1159; though, genotype 1114 showed more susceptible to heat stress with reference to yield. Seeds yield is interlinked with rate of grain filling, faster grain filling rate enhanced grain yield. This result corresponds with the conclusions of researchers including Barma (2005) and Subhani et al., (2000). On other side Modarresi et al., (2010) evaluated that heat stress minimizes yield of grain up to 46.63%. Qamar et al., (2004) suggested that wheat cultivars under optimum temperature have higher grain yield. Refay (2011) appraised three wheat lines and described 7.9% reduction in wheat grains. Our results are in according to those of Rahman et al., (2009) that found dissimilarity in grain yield per plant of numerous wheat genotypes. Our findings are also similar to Rasal et al., (2006).

During analysis of physiological traits viz. total proline contents, chlorophyll contents, membrane stability index, significant results were obtained. There was a substantial decrease in membrane stability index when all the selected germplasms were subjected to heat environment. Lowest decrease in membrane stability index was recorded in 1137. Sullivan and Ross (1979), Raison et al., (1980), Almeselmani et al., (2006) conformed that cell membrane remains efficient during stress situations and involves in adaptation to high temperature. Increase in membrane constancy in some genotypes is due to their heat tolerant genetic back. Dhanda et al., (2006) and Sikder et al., (2001) recommended that membrane thermo-stability tolerance can be utilized to find out the heat tolerance of wheat diversities. Under stress situations, accretion of proline might be because of expression of proline synthetic enzymes or due to suppressed activity of proline breakdown. In our case, more proline concentration was detected in genotypes 1123, 1124 and 1137. Ozden et al., (2009) concluded that in many plants accumulation of proline becomes increases due to abiotic stresses. However, the existing results noticeably displayed that heat stress caused deterioration in proline accumulation in wheat. Significant reduction was detected in chlorophyll contents among different genotypes. Reduction is due to the destruction of structure and function of chlorophyll. Many investigators viz. Almeselmani et al., (2006), Kaur et al., (1989), Xu (1991) and Amani et al., (1996) described that due to high temperature coverage there is an extreme decrease in chlorophyll content. Reynolds et al., (1994) indicated impulsive failure of chlorophyll due to heat compassion in wheat. Variation in carotenoids and leaf chlorophyll has been reported under maximum temperature by Bhanu (1997). Moreover, heat stress prevents biosynthesis of chlorophyll as documented by Tewari and Tripathy (1998).

Conclusion:

Tolerance to heat stress is a complex process and is controlled by various genes controlling a number of morphological and physiological changes. No single trait fully explains why some wheat varieties are able to generate better yield under heat stress. In the current research, morpho-physiological traits showed a varied response against heat stress environment and also have impact on production of wheat amongst all varieties genotypes 1067, 1123, 1124, 1137, 1154, 1159 and 1163 confirmed more tolerance to heat stress regarding to, yield per plant, 1000 grain weight, proline and chlorophyll contents of leaves. Heat tolerant genotypes should be utilized in breeding programs and wheat lines had potential to accept high temperature must be used under variety of warmer environment to improve their progress against heat stress.

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