International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182, Print ISSN: 2349-5979; Impact Factor: RJIF 5.72
Received: 03-11-2020; Accepted: 18-11-2020; Published: 06-12-2020
www.allsubjectjournal.com
Volume 7; Issue 12; 2020; Page No. 13-19

Evaluation of agronomical and physiological characters of wheat (*Triticum aestivum* L.) genotypes to heat stress

Imran Khan[1*], Sawaira Jadoon[2], Ahmad Zada[3], Amir Zaman Shah[4], Adil Altaf[5]
[1, 3] College of Bioscience and Biotechnology, Yangzhou University Yangzhou, China
[1] Department of Botany, Hazara University Mansehra KP, Pakistan
[2] School of Life Sciences, Lanzhou University, Gansu Province, China
[4] College of Horticulture and Plant Protection, Yangzhou University, Yangzhou, China
[5] College of Agriculture, Yangzhou University, Yangzhou, Jiangsu, China

Abstract
Heat stress is a major environmental factor limiting wheat productivity in most cereal growing area worldwide. This study was conducted to analyze the influence of heat stress on the performance of agronomical and physiological characteristics of wheat germplasm at NARC Islamabad during 2014- 2015. Different yield-related trait was evaluated by using CRD design with three replicate. The result indicates that extreme temperature causes reduction to grain yield and yield components such as spike length, number of grains/spike, grain weight/spike and biomass. Obtain result indicates that all genotypes responded differently against evaluated temperature as compare to optimum temperature. Among all genotypes, 1067, 1123, 1124 and 1137 confirm more tolerant to heat stress regarding grain weight/spike, the number of grain/spike/, biomass, membrane stability, and total soluble sugar content. It is suggested that heat-tolerant varieties should be utilized in a breeding program for the development of wheat varieties having heat tolerance at different growth stages of plants and more research study must be conducted to evaluate the progress of potential heat acceptable genotypes in high temp regimes.

Keywords: wheat, heat stress, yield, relative water content, sugar content

1. Introduction
Wheat (*Triticum aestivum* L.) is the most vital member of family Poaceae and serves as the main staple food crop of the world (Abd-El-Haleem *et al.*, 2009) [1]. It is a worldwide foremost cereal crop plant (Gustafson *et al*., 2009) [11]. Wheat beginning was started as part of the 'Neolithic Revolution' about 10,000 years ago, at that time earliest cultivated forms were diploid and tetraploid (Pushpalatha *et al.*, 2007) [20]. It is domesticated in the south-west of Asia since its extent to further parts of Europe, Asia, America and Africa (Bertholdsson, 2005) [5]. The optimum growing temperature for wheat has been reported between 25°C with the least and highest growth temperatures 30°C to 32°C. It is believed that in Pakistan wheat could be grown in October to December when temperature ranged 20-30°C.
Increased temperature level causes permanent harm to the development and growth of plant were called as heat stress. Typically, 10 to 15°C rise in temperature can be measured as heat shock or heat stress. Increase in temperature above the optimum level can be deleterious, causing injury to the plant were named as 'heat stress' (Wahid *et al.*, 2007) [26]. Rise in temperature is a major problem leading to a reduction in plant growth and production (Parent *et al.*, 2010) [18]. It is estimated that one-degree rise in temperature above optimum temperature for wheat growth could minimize the wheat yields about 3-10% (You *et al.*, 2009) [28]. Heat stress is a severe risk to the production of crop universally (Hall, 2000) [12]. In Pakistan during grain filling period of wheat temperature become increase and resulting in instability in wheat yield (Khan *et al.*, 2014) [15]. Temperature about 28-30°C even for 5 or 6 days can minimize 20% wheat yield because this temperature leading to reduce tillering capacity, lessening the grain filling period and accelerates crop senescence (Bahar *et al.*, 2011) [4]. Yield and yield components are still the most effective traits by stress condition (Ozkan *et al.*, 1998) [17]. Grain filling duration was also applied as a measurement to tolerate heat (Fokar *et al.*, 1998) [9]. Heat stress reduces photosynthesis (Pushpalatha *et al.*, 2007) [20], promoted senescence of flag leaves (Tewari and Tripathy, 1998) [24], decreases relative water contents (Gustafson *et al.*, 2009) [11] lessening of grains number and grain size (Freeha *et al.*, 2008) [10], reduces starch deposition (Moreno and Orellana, 2011) [16].

2. Materials and methods
Ten selected 45-IBWSN (International Bread Wheat Screening Nursery) wheat genotypes obtain from wheat wide crosses and cytogenetic lab NARC Islamabad Pakistan, the genotypes 1038, 1067, 1114, 1121, 1123, 1124, 1137,1154,1159,1163 were used in the study. the wheat genotypes were sown in pots (30 × 40 cm size) having 10 kg of loam sandy soil in a glasshouse under natural daylight at N.A.R.C. Islamabad (latitude 33.38°N, longitude 73.00°E) during the winter/spring with the average day-night temperature 30 ± 9 °C and 13± 7 °C respectively. The recommended dose was provided by NPK (120-100-60 kg ha^{-1}) was applied as urea. The pots were arranged in a complete randomized design (CRD) with three replicates. When the plant reached at anthesis stage the heat stress treatment was provided. For heat treatment one set was shifted in the glasshouse where the internal glasshouse temperature was maintained at 35- 40°C. the treatment was

provided for consecutive 7 days, daily 5 hours treatment were provided, proper irrigation was provided to heat treatment as well as normal condition treatment. By reaching maturity stage different morph-physiological traits were studied such as spike length, number of grains/spike, grain weight/spike, biomass, leaf flag area, relative water content, Carotenoids and total soluble sugar contents from different wheat genotypes at the appropriate stage to examine the variation with quantitative and qualitative traits.

2.1. Spike length
From base to the tip of spike without awns spike length of mother shoot of selected plants was measured in (cm) at last average spike length was calculated.

2.2. Number of grains per spikes
For each replication of every genotype, the spikes of the mother shoot were threshed manually and numbers of grains per spikes were counted.

2.3. Grain weight per spike
For each replication of every genotype, the spikes of the mother shoot were threshed by hand and weight of grains per spikes was measured in (g).

2.4. Biomass per plant
After harvesting the chosen plants were weighed jointly before threshing for getting their biological yield in grams and average was determined.

2.5. Flag leaf area
Maximum length and breadth were measured in centimeters per square (cm^2) from the fully developed flag leaf of selected mother shoot; the then data was noted in the morning hours when the leaf was fully turgid. Flag leaf area was calculated by using the following function of Muller (1991)

Flag leaf area = Flag leaf length × Flag leaf Width × 0.74

2.6. Relative Water Content of Leaves (RWC)
At flowering stage relative water content of leaves was taken by following the method given by Gupta (1995). Leaves taken from plants in pots were reaped. After that fresh weight harvested leaf was noted. In beaker distilled water was taken and leaves were dipped in water and left for24 hours. After that weight of fully turgid leaves were noted again.

At 70ºCfor 72hoursleaves were oven-dried, until the constant weight of leaves was calculated. By applying the following formula, the relative water content of the leaves was calculated.

RWC%= FW-DW / FTW-DW *100

2.7. Carotenoid
1ml crude leaf preparation was mixed with 10ml of 80 % ethanol and permitted to stand in the dark at room temperature for 10 minutes. Then centrifuged at 2000rpm for 5 minutes to clear the suspension. For the determination of chlorophyll supernatant, which contained soluble pigment, was used. The absorbance of the solution was read at 663 nm (chlorophyll b) and 645 nm (chlorophyll a) on spectrophotometer against 80% ethanol blank. Following the equation given by (Arnon, 1949) total chlorophyll was determined

2.8. Determination of total soluble sugar content
By phenol sulfuric acid method (Dubois *et al.*, 1956) [6] total soluble sugar content was determined0.1 gm fresh leaves were extracted with 5 ml of 80% ethanol, then in a 95°C-water bath for 10 min boil the samples in glass tubes. After extraction, for 5 min at 489 rpm, the tubes were centrifuged, and for sugar analysis, the supernatants of the extractions were used. 100µl of the sample was added to 900 µl of distilled water then mixed by vortex mixture. One ml of 5% phenol and 5 ml of H_2SO_4 were added to 1 ml of the sample and the mixture was stirred. For blank run ethanol, 80% was used. The absorbance of the sample was recorded at 490 nm after cooling at room temperature for 15 min. The concentration of the unknown sample was calculated regarding standard curve made of glucose.

Statistical analysis
For statistical analysis use different software Statistica 8.1 and SPSS.

4. Results
4.1 Spike length
The result indicates that spike length was significantly affected in treatments whereas non-significant differences were noted among most of the genotypes under normal and heat stress condition (Table 1). The LSD results showed that genotypes-1123 had minimum spike length (9.800 and 10.500cm) in both treatments respectively. The genotype-1163 had maximum (15.700cm) spike length under normal temperature however it had (11.800cm) spike length under stress condition. Reductions in spike length were observed in all genotypes at high temperature (Fig 1).

Table 1: Mean value of spike length (cm) and no. of grains spike-1 for genotypes into treatments.

S. No	Genotypes	Spike length (cm)		No. of grains spike-1	
		Normal condition	Heat stress condition	Normal condition	Heat stress condition
1	1038	13.733AB	10.500C	54.000BC	48.667CD
2	1067	12.567BC	10.600C	58.333ABC	55.667ABCD
3	1114	12BCD	12.167BC	58.00ABC	51.667BCD
4	1121	14.167AB	13.667AB	48.667C	45.000D
5	1123	9.800D	10.500C	62.667AB	60.00ABC
6	1124	10.433CD	12.767AB	54.333AB	65.667A
7	1137	14.500AB	12.900AB	65.000A	57.667ABC
8	1154	15.200A	14.500A	61.333AB	61.667AB
9	1159	13.567AB	13.433AB	62.667AB	56.000ABCD
10	1163	15.700A	11.800BC	65.667A	57.667ABC
CV		2.585	1.954	10.393	12.011

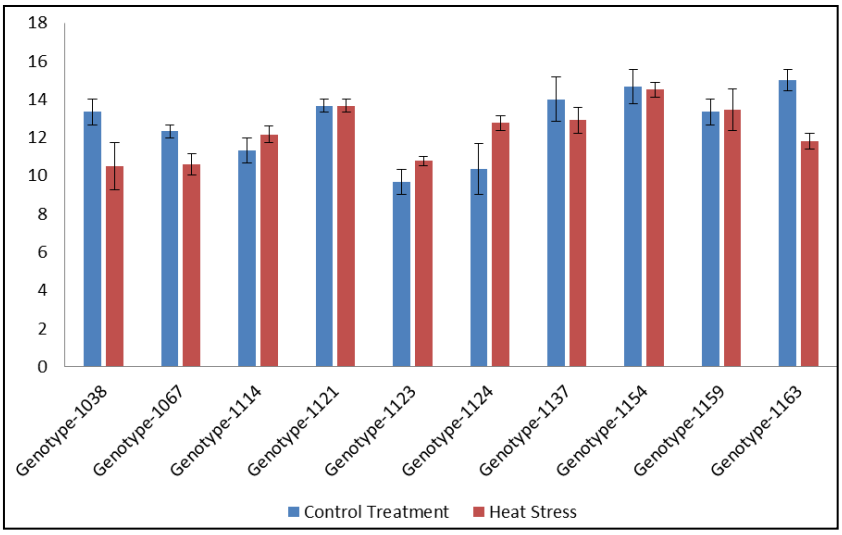

Fig 1: Spike length in normal and heat stress condition

4.2. Number of Grains per spike

Average values of grains per spike for treatments reveals non-significant variations in normal and stress conditions (Table 1.). Grains number ranged from 48.667 to 65.667 at normal temperature and under high temperature, it was from 48.667 to 61.667. The result showed that genotype-1121 had lowest grains no. (48.667 & 45) at normal and increased temperature while highest grains no. (65.667 & 57.667) were observed in genotype-1163 in normal and stress condition respectively (Fig 2). It is studied that in the majority of genotypes grains number per spike decreases under heat stress as compared to normal condition.

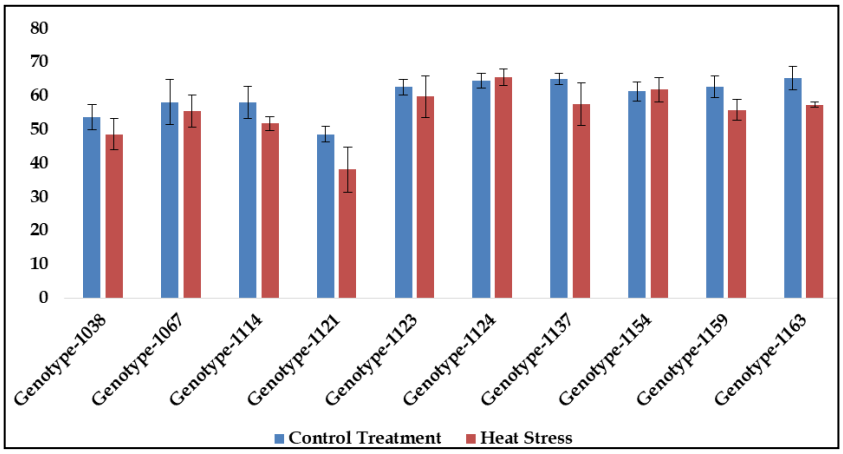

Fig 2: No. of grains per spike in normal and heat stress condition

4.3. Grain weight per spike

Results showed that HT resulted in the reduction of grains weight per spike under stress environment. Grains weights were ranges from 2.003 to 2.920g at normal temperature and under high temperature, it was from 1.333 to 2.003g. In normal condition minimum grains weight (2.003g) was observed in genotype-1124while in the same genotype it was (1.890g) under high temperature (Table 2). Among all genotypes, maximum grains weight (2.920g) was revealed in genotype-1114 at normal temperature though under stress treatment same genotype had (1.550g) grains weight per spike (Fig 3)

Table 2: Mean value of Grains weight spike-^{1}(g) and biomass for genotypes into treatments

S. No	Genotypes	Grains weight spike-^{1}(g)		Biomass (g)	
		Normal condition	Heat stress condition	Normal condition	Heat stress condition
1	1038	2.336AB	1.333C	67.170A	50.817A
2	1067	2.880A	1.676ABC	78.587A	67.167A
3	1114	2.920A	1.550BC	74.647A	49.597A
4	1121	2.066B	1.710ABC	67.347A	60.463A
5	1123	2.053B	1.850AB	65.650A	53.167A
6	1124	2.003B	1.890AB	67.793A	59.290A
7	1137	2.316AB	2.106A	71.880A	56.110A
8	1154	2.663AB	1.913AB	71.070A	60.207A
9	1159	2.403AB	2.003A	64.583A	56.413A
10	1163	2.493AB	1.983AB	79.820A	54.580A
CV		0.764	0.441	20.866	19.639

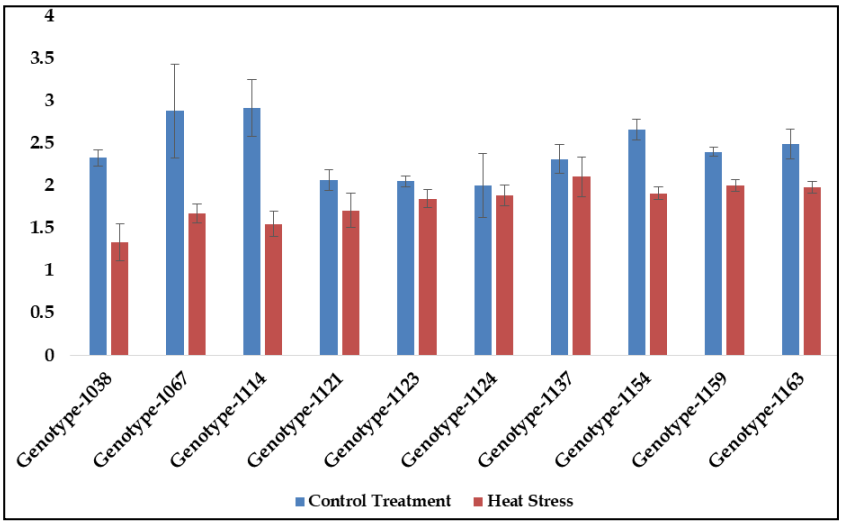

Fig 3: Grains weight per spike in normal and heat stress condition

4.4. Biomass

The LSD of biomass revealed that it was ranges from 67.170t0 79.820g at normal temperature and were 49.597 to 67.167g at high temperature. The results revealed that genotype-1159 had minimum biomass (64.583g) under normal condition but it was (56.413g) for the same germplasm in the stress environment. Maximum biomass (79.820 & 78.587g) were observed in genotype-1163 and 1067 in normal condition while under stress condition biomass of same genotypes were (54.580 & 67.167g) respectively(Table 4.2 and Fig 4).

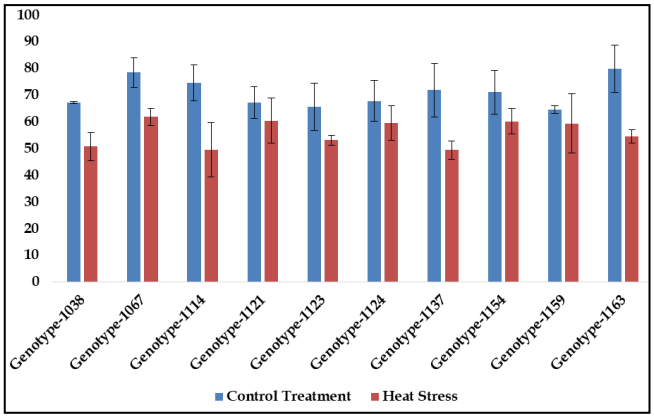

Fig 4: Biomass in normal and heat stress condition

4.5. Leaf Flag Area

The non-significant influence of treatments was recorded on leaf flag area (Table 4.3) as well as in the majority of genotypes under normal and heat treatment. The results indicate that minimum leaf flag area (27.893cm^2) was noted in genotypes-1038 under normal condition while it was (33.820cm^2) for the same wheat line under high temperature. The genotype-1114 had maximum (47.317, 40.303 cm^2) leaf flag area under normal and raised temperature (Fig 5). Under stress condition, leaf flag area in most of the genotypes comparatively reduced than normal condition.

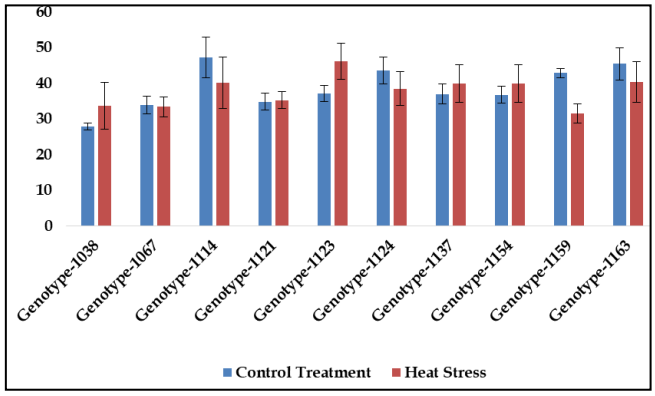

Fig 5: Leaf flag area in normal and heat stress condition

4.6. Relative Water Content of Leaves (RWC)

The table result shows the mean value of treatments which indicates significant results of relative water content. The LSD table (4.3) showed that relative water content was non-significant among the majority of genotypes under control and heat treatments. It is noted that relative water content was decreased under heat stress conditions. The relative water content was minimum (28.333%) in genotype-1154 under normal and stress conditions while it was increased (31.333%) in genotypes 1121, 1124 and 1163 under normal condition and were (30.000, 28.000 and 29.000%) at high temperature respectively (Fig 6).

Table 3: Mean value of leaves of Leaf flag area (cm^2) and RWC for genotypes into treatments

S. No	Genotypes	Leaf flag area (cm^2)		RWC (%)	
		Normal condition	Heat stress condition	Normal condition	Heat stress condition
1	1038	27.893D	33.820A	30.333AB	27.333A
2	1067	34.000CD	33.473A	29.667AB	30.000A
3	1114	47.317A	40.303A	30.000AB	29.000A
4	1121	34.900CD	34.700A	31.333AB	30.000A
5	1123	40.467ABC	39.820A	32.667A	30.000A
6	1124	43.710ABC	36.933A	31.333AB	28.000A

7	1137	37.020ABCD	39.920A	30.667AB	29.000A
8	1154	36.837BCD	39.920A	28.333B	28.333A
9	1159	42.913ABC	31.643A	30.333AB	29.000A
10	1163	45.563AB	40.333A	31.333AB	29.000A
CV		10.356	14.131	3.109	3.261

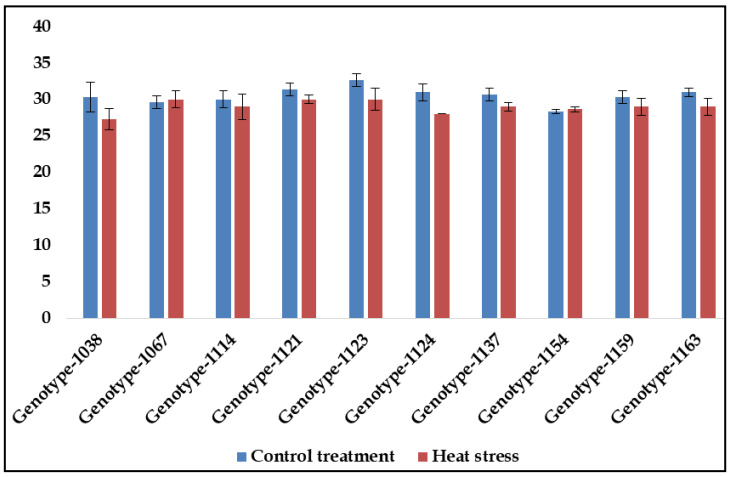

Fig 6: RWC of leaves in normal and heat stress condition

4.7. Leaves carotenoid contents

The average values showed significant variations for carotenoid contents under treatments Mean values for treatment into genotype interaction showed important differences in few genotypes and indicates that high temperature causes a decline of carotenoids in the majority of genotypes (Table 4.4). Under normal condition less carotenoid contents (0.276mg/g) were observed in genotype-1038 while it was (0.143mg/g) in stress condition. Maximum carotenoids (0.550mg/g) were detected in genotype-1163 in normal condition and it were (0.136mg/g) at high temperature in same germplasm (Fig 7).

Table 4: Mean value of Carotenoids (mg/g) and soluble sugar for genotypes into treatments

S. No	Genotypes	Carotenoids (mg/g)		Sugar contents (mg/g)	
		Normal condition	Heat stress condition	Normal condition	Heat stress condition
1	1038	Normal condition	Heat stress condition	1.967B	2.666AB
2	1067	0.276D	0.143ABCD	2.233A	3.033A
3	1114	0.366BC	0.166ABC	1.733BCD	2.866A
4	1121	0.333CD	0.176A	1.933B	2.533AB
5	1123	0.320CD	0.173AB	1.600D	2.933A
6	1124	0.380BC	1.176A	1.866BC	2.866A
7	1137	0.323CD	0.136ABCD	1.600D	1.866C
8	1154	0.446D	0.166ABC	1.633CD	2.200BC
9	1159	0.386BC	0.130CED	1.866BC	2.266BC
10	1163	0.423B	0.116D	1.633CD	1.800C
CV		0.550A	0.136BCD	0.250	0.535

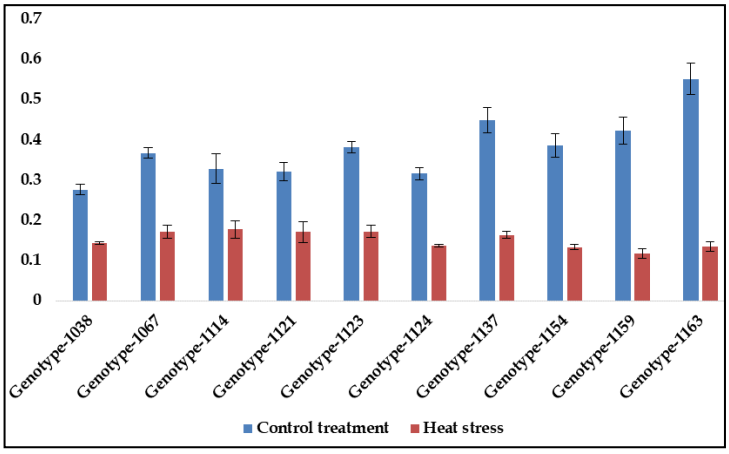

Fig 7: Concentration of carotenoid in normal and heat stress

4.8. Total soluble sugar content determination

The significant variations were observed in soluble sugar contents under normal and raised temperature Mean values for treatment into genotype interaction showed important differences for soluble sugars in some genotypes in normal conditions though non-significant variations were recorded under stress treatment (Table 4.4). It is revealed that sugar contents were decreased under the normal condition as compared to heat stress condition. Fig (8) shows detection of soluble sugar contents in genotypes. Less sugar contents (1.600 & 1.866mg/g) were observed in genotype-1137 while it was high (2.233 & 3.033mg/g) in genotype-1067respectively under both treatments.

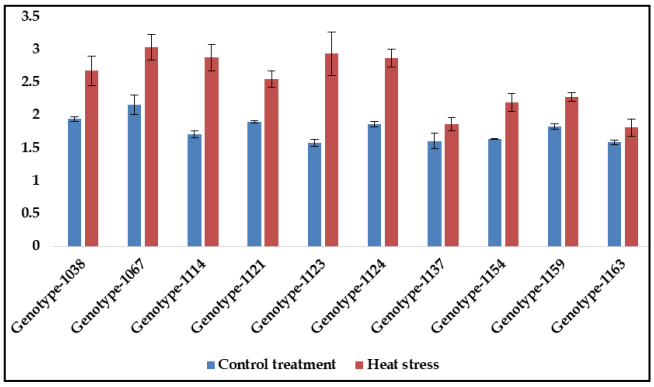

Fig 8: Concentration of soluble sugar content in normal and heat stress condition

5. Discussion

In conducting experiments spike length show significant differences under stress environment. Spike length become short due to stress as genotype 1154 had more spike length than all other selected genotypes. (Hozayn and Abd El-Monem, 2010) [13] in their finding found that reduction reached 5.00 and 6.22% for spike length. Numbers of grains per spike dropped by more temperature as in our study germplasm 1137 and 1163 showed more grains per spike as compared to all evaluated genotypes. Related effects were perceived by (Yildirim and Bahar, 2010) [27] as they observed that in heat stress condition the number of grains per spike decreased from 33 to 13. (Farooq *et al.*, 2011) [7] and (Ur Rehman *et al.*, 2009) [25] also stated such findings. In the present evaluation, it was observed that germplasm 1114 had minimum grain weight spike-^{1} while more grain weight was reported in genotype 1137 and 1159 under stress condition. Reduction in grain weight may be due to shortening of photosynthetic duration which results in less deposition of starch by injury of the starch synthesizing enzyme. Reduction of about 21 and 35% in grain weight spike-^{1}was reported by (Assad and Paulsen, 2002) [3]. Later (Shah and Paulsen, 2003) [21] found that the reduction in weight results from reduction in flag leaf area and early leaf senescence decline in wheat biomass was assessed by heat stress environment as genotypes 1067, 1121, 1154 and 1159 experienced increased biomass under stress than all evaluated varieties. Reduction in biomass might because of more fast senescence and increased respiration. Our conclusions were also supported by (Singh *et al.*, 1997) [22], (Singh and De, 1978) [23]. Many reporters directed that unfavorable temperature in wheat reduced total plant biomass by (Tewari and Tripathy, 1998) [24] Wardlaw, (1989), (Prakash, 1997) [19]. Flag leaf area is one important trait for a crop to produce dry matter and grain yield by (Fischer, 1985) [8]. There was a distinction for flag leaf area among observed genotypes both for high and optimum temperature. Simon (1999) noted reduction as a result of a raised temperature. In the present experiment, the flag leaf area was higher in 1123 and 1114 than all other wheat lines. Lessening of leaf area under high temperature was also detected by Campbell and Read (1968). Significant reductions in RWC were recorded at reproductive stages of wheat in all breed lines. This might be due to more transpiration rate under stress. In RWC extreme reduction was verified in genotypes including 1038 and 1154 while least RWC reduction was recorded in genotype 1067. Various workers including (Prakash, 1997) [19] Bhanu (1997), Misra (1990) and Deshmukh *et al*., (1991) perceived that tolerant genotypes possessed high RWC. Our finding agrees with the research of (Kesici *et al.*, 2013) [14]. Variation in carotenoids and leaf chlorophyll has been reported under maximum temperature by (Prakash, 1997) [19] Bhanu (1997). Moreover, heat stress prevents the biosynthesis of chlorophyll as documented by (Tewari and Tripathy, 1998) [24]. More sugar contents were observed in wheat varieties under stress condition than normal condition. Under stress condition increase soluble sugar contents can be a defense mechanism for the plant under stress because it can be rolled for expression of heat shock genes. Genotype 1067 showed tolerance to a high temperature by increasing sugar contents

6. Conclusion

The present study indicates that most of the wheat (Triticum aestivum L.) Genotypes were more sensitive to high-temperature stress. During the time of anthesis, most of the genotypes very highly effected. While some genotypes generate better yield during high-temperature stress. In the present study morphological agronomical and some physiological trait shows a varied response to heat stress and having a good impact on the production of wheat. Among all of the genotypes 1067, 1123, 1124 and 1137 showed more tolerance to high-temperature stress. Heat tolerant genotypes along with other contributing characters should be utilized in the breeding program and used as selection criteria in breeding programs.

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