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ANALYSIS OF MORPHO-PHYSIOLOGICAL CHANGES OCCURRING IN CHILLI GENOTYPES (*Capsicum* spp.) UNDER HIGH TEMPERATURE CONDITIONS

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Chillies (*Capsicum* spp.), the second most consumed vegetable around the world, belongs to the *Solanaceae* family. Chilli crop is negatively affected by high temperature which leads to economic yield losses in fruit production. Thus, the current study was designed with the aim to evaluate the various morpho-physiological and biochemical characters which can be useful for evaluation of heat tolerance potential in chilli genotypes and to assess the genotypes against stress. A pot experiment was conducted in growth chamber of mushroom lab of Institute of Horticultural Sciences, University of Agriculture, Faisalabad during 2015. Four chilli genotypes C-37, Uk-101, H-13 and Jawala were chosen to undergo morpho-physiological and biochemical investigations under high temperature (40/32ºC day and night temperature). Results regarding growth and physiological attributes were found effective for the assessment of heat tolerant potential in chilli crop. Further, the results also demonstrated significantly different genotypic behavior of chilli genotypes against heat stress. Conclusively, it can be stated that chilli genotype namely C-37 is found most heat tolerant among the studied genotypes and can be used to enhance chilli yield under stressful environmental conditions of elevated temperatures.
Key words: heat stress, high temperature, chilli, morphological, physiological

INTRODUCTION

Climate change induced environmental stresses affects almost all the horticultural crops. Vegetable production is potentially threatened by a variety of abiotic stressors such as heat, salt and drought stress etc. (Jain *et al.,* 2009). However, high temperature stress is severely damaged the plant growth and development which ultimately reduced the plant productivity and quality (Abbas *et al.,* 2017; Alam *et al.,* 2017; Shaheen *et al.,* 2015; Ruelland and Zachowski, 2010).
Alarming increase in temperature due to global warming has become a potential risk for production of crops around the globe (Hall, 2001). According to the report of inter-governmental panel on climate change (IPCC) an increase of summer temperature up to 3ºC in few coming years will be of a great concern due to global warming. According to the reported facts, this situation may lead to severe famines worldwide (IPCC, 2012).
Chilli (*Capsicum* spp.), the member of a well-known "the night shade" family is the second most consumed vegetable as well as spice in the world, (Howard et al. 2000; Perry *et al.,* 2007; Mahmood *et al.,* 1999). Chillies are believed to be originated from Mexico, northern South America and Central America (Patluri *et al.,* 2018; Farooqi *et al.,* 2005). Globally, chillies production exceeds 3.13 x 10^6 thousand ton major share of which is coming from china being its largest producer (FAO STAT, 2013). Chillies are grown on large scale in Pakistan as a cash crop, occupying the largest crop area after potatoes and onions (Anonymous, 2000). Pakistan is accomplishing its domestic requirements alongwith exporting enormous quantities of chillies to Gulf States and Canada adding per annum 1.127 billion rupees in GDP (1.5%) for Pakistan (Hussain and Abid, 2011).
Various scientists described the drastic effects of heat stress on vegetables. (Gruda, 2005; Abdelmageed and Gruda, 2009). Hayat *et al.,* (2009) reported that under high temperature stress each degree rise in temperature can cause 17% reduction in crop yield. Heat stress resulted in reduced photosynthesis, lessor respiration rate and disturbs plant adjustments due to perturbations of leaf respiration rates (Goraya and Asthir, 2016). Abdelmageed and Gruda, (2009) observed that high temperature stress negatively effects the pollination process, quality of fruit and viability of seeds in chillies, tomato and some other vegetable. The detailed effects of heat stress on chilli crop are not much studied until now; hence, this study was carried out in order to find out heat stress damages on various characteristics of locally available four chilli genotypes.

MATERIALS AND METHODS

The proposed research work was performed in growth chamber of mushroom lab of Institute of Horticultural Sciences, University of Agriculture, Faisalabad during 2015. While one of the experiments was conducted with four chilli genotypes, collected from Ayub Agricultural Research Institute (AARI), Faisalabad.

***Experimental details*:** Seeds of four chilli genotypes C-37, Uk-101, H-13 and Jawala as a planting material were sown in medium sized plastic pots in sand media with 4 replications (n) at 27/22 ºC day and night temperature, which were watered according to the irrigation requirement of plants. Hoagland's nutrient solution with half strength (0.5) was used for nourishment of plant. The newly emerged young, four weeks old plants were subjected to high temperature by increasing 2ºC/day temperatures up to 40/32ºC day and night temperature. Plants were kept at 40/32ºC day and night temperature for one week.

***Growth trait measurements*:** Numbers of leaves were counted manually in this study and average was obtained. Shoot length was measured using a scale. The stem was spread out on the ruler so as to ensure a straight orientation and to avoid experimental errors during measurement. Root length was also measured by using a scale in the same ways as described in case of shoot length. Roots were measured to length of the longest root hair and finally average was obtained. Weight of fresh seedling was measured by the use of an electronic, high precision weighing balance in grams. In order to get dry weight of chilli seedlings, freshly harvested seedlings were packed in labeled paper bags which were later on kept in electric oven (Memmert-110, Schawabach, Germany) at 70°C for 24 hours. After prescribed period of time, bags were taken out and weight was recorded by using weighing balance. Chlorophyll meter (CCM-200 plus Bio-Scientific USA) was used for the measurement of chlorophyll contents.

***Gaseous exchange measurements*:** Two fully developed, young, healthy and uniform sized leaves per plant (from each replication) were selected for gaseous exchange measurements like transpiration rate, photosynthetic rate and stomatal conductance to H_2O. Kept each leaf sample one by one in portable chamber of gadget named as IRGA (Infra-Red Gas Analyzer) (LCi- SD, ADC Bio-scientific UK). All the readings of physiological characteristics were determined during day time (10.00 a.m-12.00 p.m) at 99.9 kPa atmospheric pressure, 403.3 mmol $m^{-2}s^{-1}$ molar flow of air per unit leaf area, water vapor pressure in the chamber ranged from 6.0 to 8.9 mbar, photosynthetically active radiation up to 1711 pmol $m^{-2}s^{-1}$ at leaf surface, surrounding CO_2 level was 352 pmol mol^{-1} and atmospheric temperature 40°C.

Water use efficiency was measured as ratio between photosynthesis (A) and the amount of water transpired (E), formula is given below,

WUE = A/E

***Experimental design and statistical analysis*:** The experiment was conducted under complete randomized design (CRD). Analysis of variance (ANOVA) technique was employed to find the significance of the study and means were compared (Honestly significant difference Tuckey test) by using Statistix 8.1 computer software. Differences among treatments were considered significant only at $p \leq 0.01$ after statistical analysis.

RESULTS

Results of morphological attributes and physiological attributes of chilli genotypes under high temperature are given in Table 1 and Table 2.

***Number of leaves of chilli genotypes under heat stress*:** Under heat stress at 40ºC, C-37 genotype was successful in attaining the higher number of leaves followed by H-13 and Jawala. Data showed in figure 1a, represents the significant difference regarding number of leaves among chilli genotypes at 40ºC temperature (Under high temperature stress).

***Seedlings shoot length (cm) of chilli genotypes under heat stress*:** Heat stress markedly reduced shoot length in chilli crop. Genotype H-13 and Jawala, in comparison to genotypes C-37 and UK-101 showed reduced shoot lengths of seedlings. Chilli genotypes C-37 and UK-101 have shoot length of 7cm

Table 1. Comparison of morphological traits of chilli genotypes at high temperature

Chilli Genotypes	NL	SL	RL	SFW	SDW
C-37	6.75±0.25a	7.50±0.040a	6.75±0.064a	0.96±0.0095a	0.28±0.0040a
UK-101	5.75±0.25ab	6.28±0.047b	6.40±0.040a	0.71±0.0085b	0.19±0.0062b
H-13	4.00±0.40c	4.21±0.048d	3.90±0.050b	0.37±0.0070d	0.08±0.0047c
Jawala	5.0±0.40bc	4.80±0.040c	3.74±0.25b	0.42±0.0085c	0.11±0.0047c

Means sharing similar letters in a column are statistically non-significant (P>0.05)

Table 2:.Comparison of physiological traits of chilli genotypes at high temperature

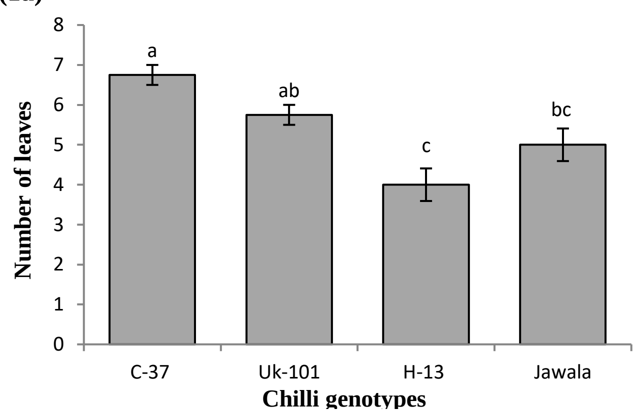
Chilli Genotypes	PR	TR	SC	WUE	CC
C-37	6.05±0.051a	1.6±0.0091c	0.78±0.0070d	3.78±0.051b	10.16±0.08b
UK-101	5.61±0.024b	1.36±0.011d	0.89±0.0063c	4.11±0.038a	11.23±0.28a
H-13	2.92±0.022c	2.34±0.0165b	1.77±0.013b	1.24±0.011c	5.70±0.04d
Jawala	2.49±0.035d	2.53±0.0064a	1.90±0.030a	0.98±0.014d	6.49±0.06c

Means sharing similar letters in a column are statistically non-significant (P>0.05) and 6.8cm, respectively as well as H-13 and Jawala have 4.2 cm and 4.8cm shoot length, respectively, under high temperature stress (Figure 1b).

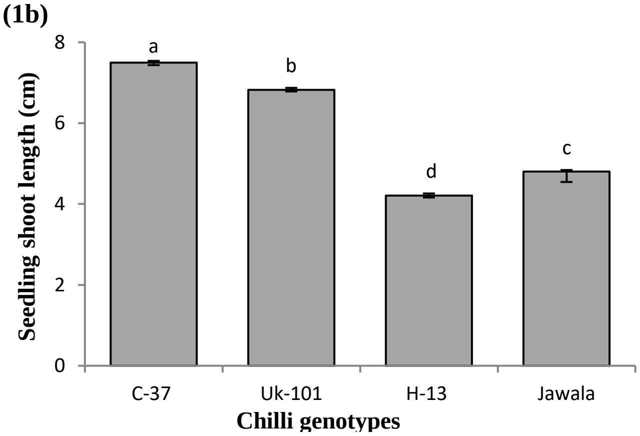
***Seedlings root length of chilli genotypes under heat stress*:** Effect of heat stress on the seedling root length of chilli genotypes is shown in figure 1c. Under high temperature conditions, chilli genotypes C-37 and UK-101 exhibited the maximum root length of 6.75cm and 6.40 cm, respectively, while genotypes H-13 and Jawala exhibited minimum root length of 3.90 cm and 3.74 cm, respectively. It can be concluded that at 40ºC temperature (high temperature stress), C-37 and UK-101 had higher values for seedling root length as compared to H-13 and jawala.

***Plant fresh weight (g) of heat tolerant and sensitive chilli genotypes under heat stress*:** All the chilli genotypes exhibited varied responses to high temperature conditions regarding plant fresh weight. Results regarding plant fresh weight exhibited higher fresh weight of 0.96 g and 0.71g in genotypes C-37 and UK-101, respectively and relatively lower plant fresh weight (0.37g and 0.42g) was found in genotypes H-13 and Jawala, respectively. Significant ($p \leq 0.01$) difference was recorded (Figure 1d) in plant fresh weight at high temperature (40ºC) among chilli genotypes C-37, UK-101, H-13 and Jawala.

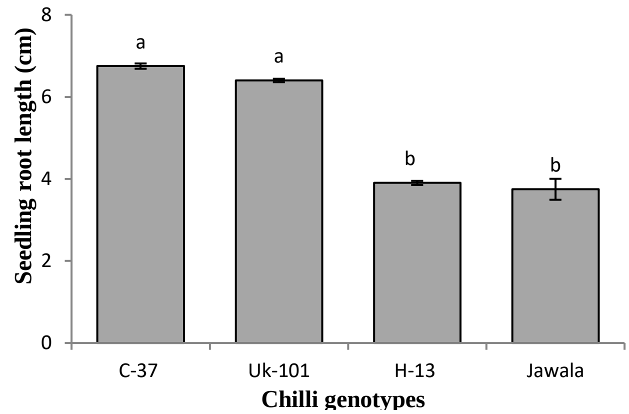
(1a)



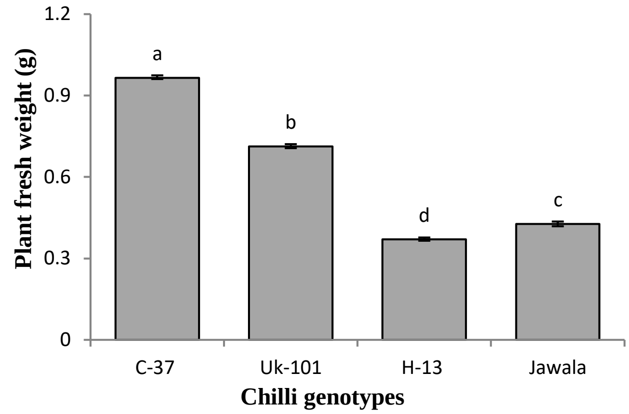
(1b)



(1c)



(1d)



(1e)

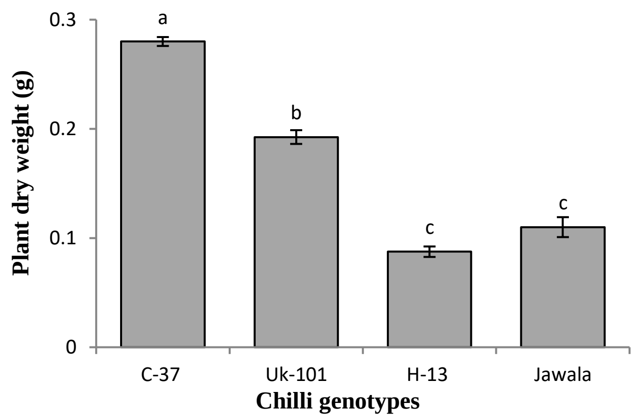


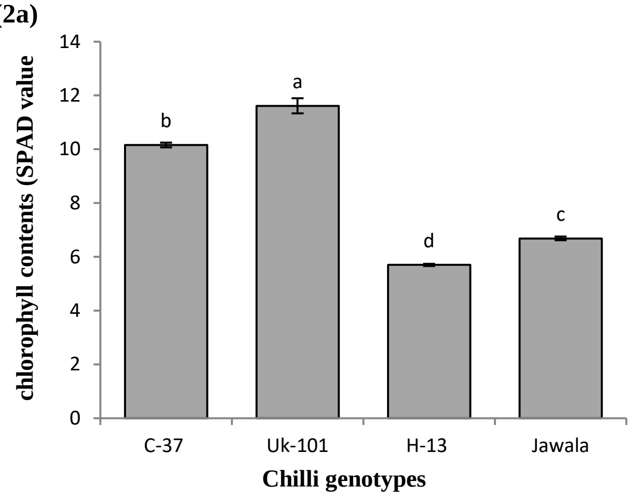
Figure 1a,b,c,d.e: Comparison of morphological traits of chilli genotypes at high temperature

***Plant dry weight (g) of chilli genotypes under heat stress*:** Results regarding plant dry weight exhibited higher dry weight of 0.28 g and 0.19 g in genotypes C-37 and UK-101, respectively and lower plant dry weight (0.087 g and0.11g) was recorded in genotypes H-13 and Jawala, respectively, under high temperature stress (Figure 1e).

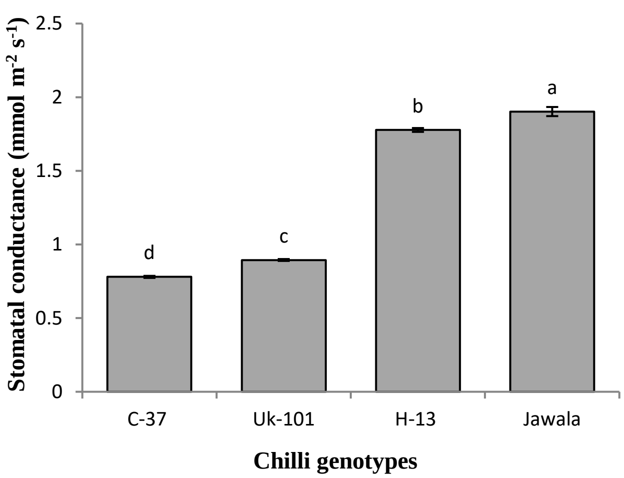
***Chlorophyll contents (SPAD Reading) of chilli genotypes under heat stress*:** The comparison among chilli genotypes revealed that they responded differently at high temperature stress (40°C) having varying values for chlorophyll contents. Chilli genotypes C-37 and UK-101 showed good performance having little heat effect on chlorophyll contents with values of 10.16 and 11.61, respectively, while genotypes H-13 and Jawala showed the poor performance for chlorophyll contents (5.70 and 6.68, respectively) as shown in figure 2a.

***Stomatal conductance (mmol $m^{-2}s^{-1}$) of chilli genotypes under heat stress*:** Plants grown under high temperature, statistically showed (figure 2b) that chilli genotypes C-37 and UK-101 exhibited the minimum stomatal conductance of 0.78 and 0.89 mmol $m^{-2}s^{-1}$, respectively, while genotypes H-13 and Jawala exhibited maximum stomatal conductance of 1.77 and 1.90 mmol $m^{-2}s^{-1}$, respectively. It can be concluded that at 40°C temperature (high temperature stress), there was a significant difference in stomatal conductance of chilli genotypes C-37 and UK-101 as compared with H-13 and Jawala.

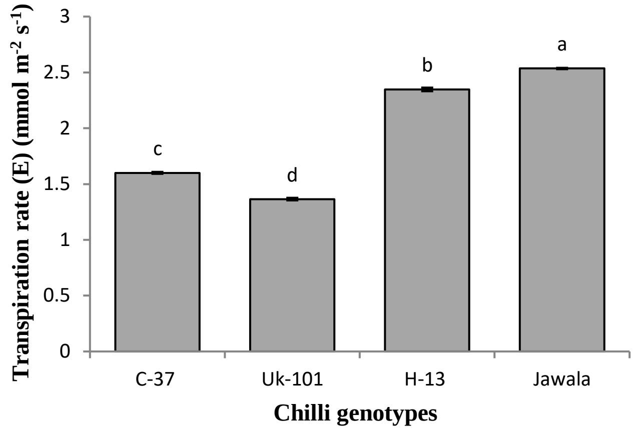
(2a)



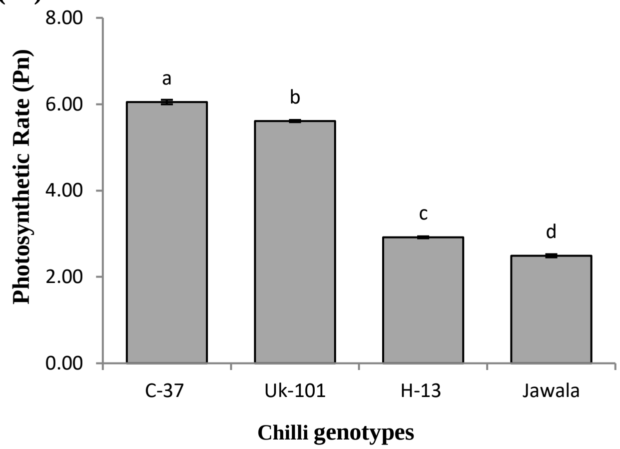
(2b)



(2c)



(2d)



(2e)

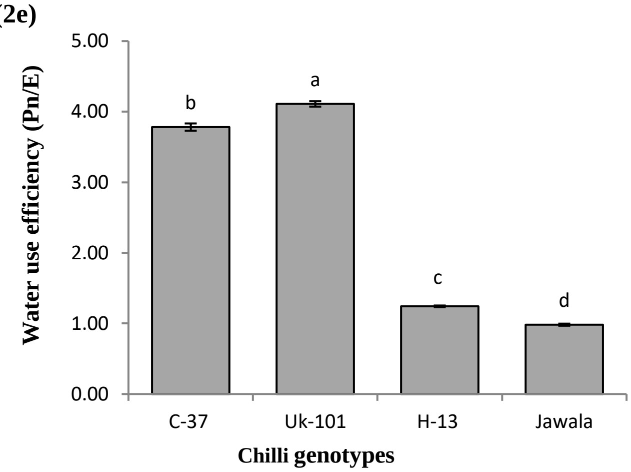


Figure 2a,b,c,d,e: Comparison of physiological traits of chilli genotypes at high temperature

***Transpiration rate (E) (mmol $m^{-2}s^{-1}$) of chilli genotypes under heat stress*:** Data regarding transpiration rate (E) in figure 2c showed that genotypes C-37 and UK-101 exhibited the minimum transpiration rate (E) of 1.60 and 1.36 mmol

$m^{-2}s^{-1}$, respectively, while genotypes H-13 and Jawala exhibited maximum transpiration rate (E) 2.34 and 2.53 mmol $m^{-2}s^{-1}$, respectively. It can be concluded that at 40ºC temperature (high temperature stress), there was a significant difference in transpiration rate (E) among genotypes C-37, UK-101, H-13 and Jawala.

***Photosynthesis rate (Pn) (µmol $m^{-2}s^{-1}$) of chilli genotypes under heat stress*:** Data regarding photosynthesis rate (Pn) in figure 2d showed that genotypes C-37 and UK-101 showed good performance with maximum photosynthesis rate (Pn) of 6.05 and 5.61 µmol $m^{-2}s^{-1}$, respectively, genotypes H-13 and Jawala exhibited relatively poor performance with minimum photosynthesis rate (Pn) of 2.92 and 2.49 µmol $m^{-2}s^{-1,}$ respectively. It can be concluded that at 40ºC temperature (high temperature stress), there was a significant difference in photosynthesis rate (Pn) among chilli genotyes C-37 and UK-101, H-13 and Jawala.

***Water use efficiency (µmol CO_2mmol^{-1} H_2O) of of chilli genotypes under heat stress*:** Data regarding the water use efficiency (Figure 2e) reflects that chilli genotypes C-37 and UK-101 exhibited the maximum water use efficiency of 3.78 and 4.11, respectively, while dgenotypes H-13 and Jawala exhibited minimum water use efficiency of 1.24 and 0.98, respectively under high temperature (40ºC) conditions. There was a significant difference in water use efficiency (Pn/E) among genotypes C-37 and UK-101, H-13 and Jawala.

DISCUSSION

Shaheen *et al.,* 2016 and Salah and Tardieu, (1996) indicated that growth of various parts of plant especially leaves were affected by high temperature conditions. These findings correlate with the results of current study that showed the significant changes among genotypic response towards morphological attributes under high temperature conditions. Chilli genotypes with higher number of leaves were found more capable for surviving under high temperature conditions as compared to genotypes which produced lower number of leaves under high temperature. However, more number of leaves indicated that higher photosynthesis and carbon assimilation rate which enhanced all the growth characteristics of chilli plants. The present study also revealed a strongly positive correlation of number of leaves with root and shoots length, seedlings fresh and dry weight and chlorophyll contents. It depicts that the morphological attributes are interlinked and cultivars that have ability to sustain net carbon assimilation rate under stressful conditions would be able to produce greater plant yield.

Shoot length exhibited highly positive correlation with seedling fresh weight and seedling dry weight. These enormous differences in genotypes depicted the heat tolerance of genotypes to enhance plant biomass under high temperature stress. These findings are similar with the results of Bakker and Uffelen, (1998) who reported the significant dependence of plant height on amplitude of day and night temperature. Kumar et al. (2005) observed decrement in plant shoot and root growth due to osmotic effect, less water absorption and low metabolic activities under high temperature stress. Rylski, (1986) worked on sweet pepper and obtained maximum plant height in screen house as compared with field conditions due to higher temperature levels in field conditions. This can be concluded that chilli genotypes with greater root length have ability to uptake water and nutrients more efficiently which are sufficient for growth and development of plants. The findings for plant root length were in agreement with the reported results of Kumar et al. (2005). These findings are correlated with the findings of Zhang et al. (2013) who reported that seedling stage was highly affected by heat stress than the fully developed ones.

Chlorophyll contents plays important role in plants and give quantitative information about photosynthesis (Maxwell *et al.,* 2000). Higher chlorophyll contents were found in C-37 while Jawala showed the lowest value for chlorophyll contents. These findings are in accordance to the results of Reda and Mandoura, (2011) who stated that chlorophyll biosynthesis was significantly reduced when plants were exposed to high temperature stress. Similar findings were also reported by Efeoglu and Terzioglu, (2009) and Balouchi, (2010). A number of reports indicating reduction in chlorophyll contents under stressful condiotions have been documented on several crops such as alfalfa (Winicov and Seemann, 1990), sunflower (Ashraf and Sultana, 2000, Akram and Ashraf, 2011) and wheat (Arfan *et al.,* 2007, Perveen *et al.,* 2010).

Gas exchange attributes (photosynthesis rate, stomatal conductance to water, transpiration rate and water use efficiency) are influenced by various abiotic factors such as extreme temperature, salt and drought stress (Delfine *et al.,* 1999). Osmotic stress is originated in plant tissues which decreased roots hydraulic conductance and water status of tissues under heat stress (Morales et al. 2003; Shaheen *et al.,* 2015). Numerous physiological processes such as translocation and ionic uptake, organic solutes, photosynthesis, respiration, evapo-transpiration, leaf osmotic potential and chlorophyll contents are severely affected by heat stress (Huve *et al.,* 2005; Taiz and Zeiger, 2006).Transpiration rate (E) showed significant difference at 40ºC temperature (high temperature stress) among the genotypes. This is in agreement with the results of Berry and Bjorkman, (1980) who found that transpiration rate is temperature dependent. Heat stress restricts the plant growth and causes dehydration in tissues and organ as a result rapid water loss from plant surface (Mazorra *et al.,* 2002).

In current investigation, it can be concluded that at 40ºC temperature (high temperature stress) had significant difference in photosynthesis rate (Pn) among chilli genotypes. Same findings were documented in many earlier reports such as photosynthetic rate is considered as one of the main indicator of heat tolerance in various crop species such as melon (Kitroongruang *et al.,* 1992) and tomato (Camejo *et al.,* 2005, Singh *et al.,* 2005). However, photosynthesis rate is heat sensitive process (Guilioni *et al.,* 2003; Wang *et al.,* 2010; Centritto *et al.,* 2011) and chloroplast plays key role in the photosynthetic machinery. Both dark and light reactions cycles occurred in chloroplast and it is also helpful for modulation of stress responses. High temperature stress causes the disturbance of thylakoid membrane by stopping the enzymes activites and actions of electron carriers, which ultimately reduced the plant photosynthesis rate (Ristic *et al.,* 2008; Rexroth *et al.,* 2011). In addition, several studies indicated that photosynthetic rate and final crop yield has positive correlation to each other (Ashraf *et al.,* 2007; Makino, 2011). In the present research there was a highly negative correlation between water use efficiency and transpiration rate, but a strongly positive correlation was there between stomatal conductance to H_2O and water use efficiency. Due to higher transpiration rate, excessive water loss occurred in the plants which decreased the water use efficiency. The regulation of stomatal conductance to H_2O plays key role in plants as it is necessary for prevention of CO_2 acquisition and desiccation (Dodd, 2003; Medici *et al.,* 2007). These findings are correlated with the current study in which lower stomatal conductance to H_2O was observed in chilli genotypes C-37 followed by UK-101, while higher found in genotype H-13 and Jawala. Under stressful environments, reduction in stomatal conductance has shielding effects because it permits the plant to save water and to enhance the water use efficiency (Chaves *et al.,* 2009). However, Camposa et al. (2014) found that stomatal closure occurred as a main limiting factor of photosynthesis in *capsicum annuum* plants. Water use efficiency of a cultivar is a main indication of its heat tolerance potential. In this investigation, there was a significant difference in water use efficiency (Pn/E) of chilli genotypes under high temperature stress. Several reports indicated that drought stress at initial stages caused the closure of stomata which ultimately increased the water use efficiency. Stomatal closure is recognized as it has more inhibitory effect on transpiration rate than CO_2 diffusion into the leaf tissues (Chaves *et al.,* 2009; Sikuku *et al.,* 2010).

***Conclusion*:** Based on the observations made during the study, it may be concluded that chilli genotypes significantly differed in response to heat stress. Chilli genotypes C-37 and UK-101 were found heat tolerant whereas, H-13 and Jawala may be taken as heat sensitive. In addition, morphological and physiological attributes proved to be of worth consideration while studying heat stress in chillies.

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