INDIVIDUAL AND COMBINED EFFECT OF TERMINAL DROUGHT AND HEAT STRESS ON ALLOMETRIC GROWTH, GRAIN YIELD AND QUALITY OF BREAD WHEAT

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Abstract

Among the abiotic stresses, terminal drought and heat stress are two critical threats to crop production worldwide. The objective of this study was to quantify the separate and combined effects of drought and heat stress (during heading and grain-filling stages) on allometric growth, yield, yield components, grain characteristics and grain quality of bread wheat. Experimental treatments were comprised of control (well-watered/normal condition), drought stress (50% field capacity), heat stress (inside the plastic tunnel) and drought + heat (50% field capacity and inside the plastic tunnels). Heat and drought + heat were found to have a much greater influence on the leaf area index, leaf area duration, crop growth rate and net assimilation of wheat plants than drought stress alone and control treatment. More reduction in yield and yield components of wheat was noted where combined stress (drought and heat) was applied. Moreover, heat and heat+drought stress reduced both the grain growth duration and the grain growth rate that decreased the 100 grain weight of wheat. A significant reduction in chlorophyll contents while higher concentration of proline and soluble protein of flag leaf over control was observed after drought heat stress treatment. In addition, quality traits of wheat grain were significantly affected under drought, heat and drought +heat stress conditions. Heat and drought +heat stress than drought alone and control treatment. It was concluded that effect of simultaneously applied drought and heat stress is more severe as compared to individual effect drought and heat stress.

Keys words: Drought, Heat stress, Grain quality, Proline, Grain growth duration, Yield and yield components.

Introduction

In the upcoming era, world is challenged due to increase in food demand leading to severe risk of food security which is aggravated by shortage of water and elevated temperature ranges day by day in changing climate (Myers et al., 2017). Crop productivity is being reduced due to increase in global temperatures, changes in the distribution of rainfall and increased drought in arid and semi-arid regions because of current scenario of climate change (Knapp et al., 2016; Schlaepfer et al., 2017). Impact of water stress on growth and development of different crop plants is diverse naturally (Jaleel et al., 2009). It might be due to reduced cell expansion and less biomass production (Basu et al., 2016), changes in various metabolic processes of the plants, reduced enzymatic antioxidants activities, ionic imbalance, instabilities in solute accumulation or a combined effect of all these factors (Farooq et al., 2009; Esmaeilpour et al., 2016). Inadequate availability of water during crop growth stages especially at reproductive stages causes harmful effects on crop productivity (Farooq et al., 2014). Nutrient uptake, unloading mechanism and lower transpiration flow can be seen in result of less absorption of inorganic nutrients (Wu et al., 2018). Basic disadvantage of moisture stress is lowered leaf water status (Esmaeilpour et al., 2016).

The increased temperature at the far ahead phases of crop period starting from pre-heading to post-anthesis must be understood as chief yield limiting feature

(Farooq et al., 2011; Maçãs et al., 2017). The most favorable temperature for wheat at grain filling stage is 20°C in sub-tropical regions as depicted by various researchers (Dwivedi et al., 2017). Yield of wheat crop is reduced up to3-4% in agriculture systems due to slight increase even 1°C from the optimum ranges of temperature at reproductive stages (Nawaz et al., 2013; Narayanan, 2018). An increase in temperature of 6-8°C during grain filling stage adversely affects grain development and grain filling duration period (Dias & Lidon, 2009). Similarly, it was observed that even short duration of high temperature (35-40°C) during grain filling had adverse effects on crop yield (Ciaffi et al., 1996; Yin et al., 2009). High temperature adversely affect normal physiological processes occurring in cell like rapid cell division, elongation and cell differentiation, particularly at cellular membranes. Effect of high temperature on wheat growth and development becomes complex after anthesis, as with optimum temperature (20-25°C), there is long period of assimilates accumulation and with high temperature (>32°C), period of assimilate accumulation becomes short which results in lower yields (Funaba et al., 2006). Due to heat and drought stress wheat is greatly affected in terms of yield losses. These two problems usually happened same time in field. The combined effect of these two stresses is most observed on wheat and cause great losses in yield (Pradhan et al., 2012). Moreover, plant metabolism may also alter due to combined effects of heat and drought as compared to single stress impacts (Zhang et al., 2010).

Effects of different abiotic stresses particularly these of drought and heat have been explored individually. Although, under field conditions, both of these stresses affect simultaneously. Combined or interactive impacts of different stresses on various crops have received far less attention. Combined impact of different stresses result in more deleterious effects such that the impact enhances more than the simple effects alone. Therefore; current study was planned and executed in order to explore the impacts of heat and drought stresses separately and in combination on allometric growth, grain characteristics and yield of wheat crop.

Materials and Methods

Plant materials and experimental treatments: A pot study was done to assess the influence of drought alone or in combination with heat stress on wheat seedlings. Homogenous lot of wheat seeds variety Faisalabad-2008, were obtained from Agronomic Research Station KarorLali Ehsan Layyah, Pakistan. The 0.1% (w/v) sodium dodecyl solution was used to sterilize the wheat seeds and then washed thoroughly usingsterilized deionized water. The seeds were placed in a clean petri dish comprising 2 sheets of sterilized filter paper moisturized with distilled water and permitted to grow in the shade at 24°C for 5 days; selected seedlings of same size and potency were transplanted to earthen pots containing12 kg of well crushed and sifted soil. The NPK was applied to wheat seedlings in required quantity in order to maintain the growth. Basal dose of phosphorus (P2O5) as di-ammonium phosphate at the rate of 90 mg kg⁻¹, potassium (K₂O) as potassium sulphate at the rate of 60 mg kg⁻¹while nitrogen fertilizer as urea at the rate of 100 mg kg⁻¹was supplemented and mixed thoroughly. Five wheat seedlings were maintained in each pot. All pots were kept under normal condition till heading stage. Drought and heat stresses were imposed at heading and grain filling stage when 50% plants initiated heading. Experimental treatments were comprised of control (well-watered and normal condition), drought stress (50% field capacity), heat stress (inside the plastic tunnel) and drought + heat (50% field capacity and inside the plastic tunnels).

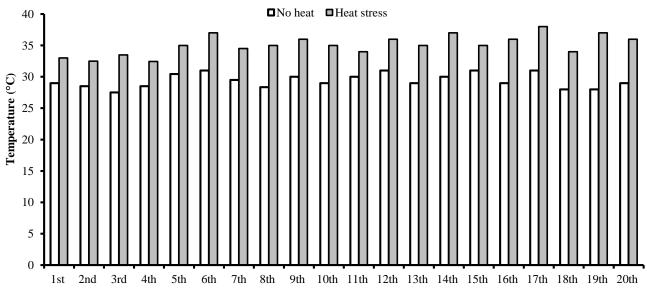
Imposition of drought stress treatment: The drought stress was imposed by maintaining the field capacity (FC). The FC was measured on a gravimetric basis (Nachabe, 1998). Gravimetric procedure of direct soil water measurement was applied to determine the water contents in the soil. Soil sampling for soil moisture measurement was carried out regularly on alternate days keeping in view the weather conditions. Composite soil samples were taken on taking into consideration for moisture determination, as the maximum moisture extraction depth of root zone of crop was taken.

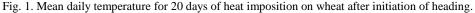
By calculating the water used for saturating the paste, field capacity was determined by the following formula:

FC = Saturation percentage of soil sample/2

During the pot experiment, the field capacity was maintained carefully for each treatment. With the help of moisture meter, moisture content of each pot was calculated every day and difference was levelled by irrigating the pot according to the calculation. This method was continued till the measurement of parameters from the seedlings.

Imposition of heat stress treatment: Heat stress was imposed at heading stage of wheat. In this regard, a plastic tunnel framework was made above desired pots, by using bamboos, enclosed within crystal clear sheet of polythene. In order to lessen moisture difference, tiny holes were made in polythene. While, controlled pots were placed in normal conditions (Shahid *et al.*, 2017b). Digital temperature and humidity probe (Digital Multimeter-50302) was used to note temperature and humidity. When provisioning the heat stress, the temperature of optimumconditioned as well as heat-stressed pots was noted twice a day and then average was taken (Both *et al.*, 2015). The significantincrease in temperature of heat-stressed pots was noted in comparison to controlled pots (Fig. 1).





Allometric attributes: From each pot, randomly selected plant samples were taken. Electric balance was used to weigh the leaves. A 5 g sample was taken from everylot of leaf. Digital leaf area meter (JVC TK-5310) was used to determine the leaf area while by taking the ratio of leaf area to ground surface area, leaf area index was measured.

Leaf area duration was calculated by using formula given below (Reddy, 2004).

$$LAD = \frac{(LA_2 + LAI_1) \times (t_2 - t_1)}{2}$$

where leaf area are indicated by LAI_2 and LAI_1 taken at time t_2 and t_1 , respectively

In order to estimate the crop growth rate (CGR) randomly selected plant from each pot were harvested and oven dried to obtain constant dry weight. By using formula given below, crop growth rate was calculated (Reddy, 2004).

$$CGR = (W_2 - W_1) / (T_2 - T_1)$$

 W_1 = oven dried weight at first sampling W_2 = oven dried weight at second sampling T_1 = time of first sampling T_2 = time of second sampling

Following formula was used to determine NAR

$$NAR = TDM/LAD$$

where,

TDM = Total dry matter accumulated (W₂ - W₁)

$$LAD = (LAI_1 + LAI_2) \times (T_2 - T_1) / 2$$

Agronomic and yield-related traits: At maturity, plant height and spike length of individually selected plant from each pot was calculated from surface of soil to ear tip, by using meter rod. The number of fertile tillers per plant, from each pot was counted when plant was matured. Harvesting and threshing of plants was done manually in order to count the spikelets numbers for each spike, grain numbers per spike, weight of 100 grain (g), and yield of grains for each plant (g). Grain-filling period was calculated according to Richards (1959) procedure. To determine the grain filling rate, three samples of spike were collected after emergence of heading with a three days interval and oven dried. Using following formula, grain filling rate (GFR) was calculated:

$$GFR = (W_2 - W_1) / (T_2 - T_1)$$

Chemical analysis: Micro-Kjeldahl method was used to determine the total nitrogen of the wheat grain sample as defined by Jackson (1973) and then by multiplying the standard factor, the percentage of grain's protein was measured. Starch and gluten contents were determined from the wheat grains according to methods described in Anon., (2000).Total soluble proteins were determined by following the Bradford assay method (Bradford, 1976). Proline was determined as described by Simaei *et al.*, (2011).

Statistical analysis: Data collected on all parameters were analyzed statistically by using Fisher's Analysis of Variance technique. Least Significant Difference (LSD) test at 5% probability level was applied to compare the treatment means (Steel *et al.*, 1997).

Results

Results showed that extreme weather conditions (water deficiency and high temperature) were capable of reducing allometric traits of wheat plants as shown in Table 1. The variances among allometric traits (leaf area duration and index, net assimilation rate and crop growth rate) were not meaningfully influenced by alone applied heat and drought stress, while greatly exposed to stress with their combination heat and drought as compared to control where stress was not imposed. As shown in Table 1, the heat + drought stresses caused much severe changes than individual effect of drought and heat stress.

The leaf area index (LAI) was decreased with imposition of stresses. Wheat plants under either of the heat and drought stress conditions promptly decreased leaf area index as compared with the well-watered controlled treatment (Table 1). Stresses assisted to decline wheat LAI either under drought and heat and drought + heat compared to controlled condition (Table 1). Crop growth rate (CGR) and leaf area duration (LAD) also decreased linearly till the end of wheat crop (Table 1). The negative effect was observed on the LAD and CGR of wheat under the drought, heat and drought + heat as compared to control; however, the effect was pronounced for controlled treatment under well-watered conditions and optimal temperature. In comparison with solely apply heat and drought stresses, their combination (drought + heat) had a harshly negative affect on both LAD and CGRand decreased 38% and 31%, respectively as compared with control (Table 1).

The plant height and number of productive tillers, the alone drought stress decreased about 25and 32%, and heat decreased 24 and 34%, while the sum of heat stress and drought significantly decreased height of plant and productive tillers 43 and 41% respectively as compared to controlled (Table 2). The similar trend were observed in case of wheat grains characteristic such as grain numbers for each spike, weight of 100 grains (g) and grains filling rate (mg day⁻¹) that were reduced by 13, 22 and 19% by drought, and by heat stress 28, 39 and 30%, while the combined stress prominently reduced about 35, 52 and 34%, respectively as compared to controlled treatment (Table 3). According to results depicted in Table 3, the same reduction pattern was observed with addition of applied stresses on the other grains yield and characterizes. Although, reduction change difference in wheat grains yield and their characteristics in response amongst drought and heat stresses alone was notes extreme, it was nevertheless significant.

Overall, yield reduction was noticed when increasing cereal wheat grains were exposed to heat stress and drought & can also be ascribed to final starch content. Resultantly, the starch synthesis was slow down with intensity of stresses. Drought and heat stress independently reduces grain starch content 10% and 19%, respectively, while combined drought + heat decreased the final starch content from grains about 21% as compared to control (Table 4).

On contrary, the with applied stresses, the prominent increased occurred in grains protein and gluten contents with increasing reduction of yield and accumulation of starch content as shown in Table 4. The highest increase in protein (40%) and gluten content (24%) was noticed for combined drought and heat stress (Table 4). Whereas, substantial difference was also observed in increasing protein and gluten contents with implication of independently drought and heat stresses (Table 4). The result depicted in Table 5 stated that environmental stress influenced the amounts of the chlorophyll content in leaves of wheat plants. The decreasing values of chlorophyll content were more sensitive to stresses and greatly influenced with combined drought and heat stress and values reduced about 30% and 42%, respectively as compared to non-stress plants. Wheat plants exposed to heat stress reduced the chlorophyll content about 20%. Whereas, solely drought stress decreased the chlorophyll content from plants leaves about 18% (Table 5).

Discussion

Abiotic stresses (especially drought and heat stress) are major environmental issues that can prominently

affect plant productivity (Lipiec *et al.*, 2013) particularly under arid region. Heat and drought stresses often correspond to brutallylimitthe growth, yield and productivity of wheat (Grigorova *et al.*, 2012; Lipiec *et al.*, 2013). While, the sum of heat and drought stressmoreseverely destruct the yield of various crops instead of their individual effect of each stress (Rollins *et al.*, 2013). Furthermore, continuous exposure of these stresses to plants can expressively alter the plant biochemistry and metabolism (Farooq *et al.*, 2009).

The results of current experiment showed the prominent effects of heat, drought and their collective application of heat and drought on wheat allometric, productivity and yield related traits. Results highlighted that impact of collective drought + heat stress upon premeditated traits wassturdieras compared to effect of separate stress. All yield and growth related parameters of wheat measured in present trial described a synergistic effect to combined drought and heat. The more destructive effect was observed on overall wheat productivity and yield instead of individual applied stress and our results are highly in accordance with Shah & Paulsen (2003), Mahrookashan et al., (2017), Balla et al., (2011) and who stated that interaction among drought and heat stress was more distinct, and consequences of combined stress on all growth, production and yield related traits were more severe than control (no stress) and individual heat and drought stress.

 Table 1. Effect of individual and combined impact of droughtand heat stress on allometriccharacters of wheat.

| | Leaf area index | Leaf area duration (days) | Crop growth rate (g m ⁻² day ⁻¹) | Net assimilation rate (g m ⁻² day ⁻¹) |
|------------------------|-----------------------------|--------------------------------|--|---|
| Control | 3.31 ± 0.191 a | 121.30 ± 4.03 a | 12.79 ± 0.75 a | 8.04 ± 0.47 a |
| Drought | $2.22\pm0.098~b$ | $82.31 \pm 5.12 \text{ b}$ | $9.67\pm0.56~b$ | $6.67\pm0.44~b$ |
| Heat | $2.17 \pm 0.090 \text{ bc}$ | $81.00 \pm 3.85 \text{ b}$ | $9.34\pm0.39~b$ | $6.42\pm0.50~b$ |
| Drought + heat | $1.92 \pm 0.092 \text{ c}$ | 75.67 ± 3.54 b | $8.80\pm0.22~b$ | $5.23 \pm 0.24 \text{ c}$ |
| LSD≤0.05 | 0.27 | 9.36 | 1.15 | 7.61 |
| CV | 6.16 | 5.52 | 6.03 | 0.944 |
| Different small letter | indicated that the means | ara significantly different (n | (0.05) Values represent me | n + SE(n-2) |

Different small letters indicated that the means are significantly different ($p \le 0.05$), Values represent mean \pm SE (n =3)

| Table 2. Effect of individual and combined impact of drought and heat stress on production | | | | |
|--|--|--|--|--|
| contributing characters of wheat | | | | |

| | Plant height (cm) | Number of productive tillers plant ⁻¹ | Spike length (cm) | Number of spikelet per spike |
|----------------|----------------------|---|---------------------------|---------------------------------|
| Control | 97.66 ± 1.30 a | 3.93 ± 0.22 a | 14.03 ± 0.96 a | $18.40 \pm 0.80 a$ |
| Drought | 73.56 ± 2.42 b | $2.97 \pm 0.39 \text{ b}$ | $9.67 \pm 0.52 \text{ b}$ | $15.19\pm0.66~b$ |
| Heat | 65.91 ± 2.65 c | 2.59 ± 0.35 b | 8.72 ± 0.48 bc | $14.85 \pm 0.51 \text{ b}$ |
| Drought + heat | 55.48 ± 2.43 d | $2.27 \pm 0.48 \text{ b}$ | 7.60 ± 0.47 c | 12.58 ± 0.89 c |
| LSD≤0.05 | 4.99 | 0.82 | 1.42 | 1.61 |
| CV | 3.26 | 14.98 | 7.54 | 5.67 |

Different small letters indicated that the means are significantly different ($p \le 0.05$), Values represent mean \pm SE (n =3)

Table 3. Effect of individual and combined effect of drought and heat stress on grain characteristic of wheat.

| | Number of grains | 100-grain weight | Grain filling rate | Grain filling duration |
|----------------|----------------------------|---------------------------|----------------------------|-------------------------|
| | per spike | (g) | (mg day ⁻¹) | (days) |
| Control | 45.32 ± 1.35 a | 4.62 ± 0.41 a | $1.85 \pm 0.070 \text{ a}$ | 27.33 ± 1.30 a |
| Drought | $39.17\pm1.92~b$ | $3.60 \pm 0.34 \text{ b}$ | $1.49 \pm 0.039 \text{ b}$ | $24.00\pm~0.85~ab$ |
| Heat | $32.64 \pm 1.10 \text{ c}$ | $2.83 \pm 0.12 \text{ c}$ | $1.29 \pm 0.038 \text{ c}$ | $21.33\pm\ 0.98\ b$ |
| Drought + heat | $29.49 \pm 1.88 \text{ c}$ | $2.20 \pm 0.09 \text{ d}$ | 1.21 ±0.035 c | $21.61\pm1.30~\text{b}$ |
| LSD≤0.05 | 3.53 | 0.61 | 0.104 | 3.762 |
| CV (%) | 5.12 | 9.90 | 3.81 | 8.95 |

Different small letters indicated that the means are significantly different ($p \le 0.05$), Values represent mean \pm SE (n =3)

| composition of wheat grain. | | | | |
|-----------------------------|------------------------------|-------------------------|----------------------------|---------------------|
| | Grain yield per plant (g) | Grain protein (%) | Grain starch (%) | Grain gluten (%) |
| Control | 4.59 ± 0.28 a | $8.82\pm0.24~c$ | 74.66 ± 3.02 a | $13.78 \pm c$ |
| Drought | $3.53\pm0.99~b$ | $11.39\pm0.56~\text{b}$ | $66.87\pm2.02~b$ | $15.95\pm b$ |
| Heat | $3.45\pm0.18\ b$ | 13.41 ± 0.67 a | 59.75 ± 1.13 c | $16.26 \pm b$ |
| Drought + heat | $3.08 \pm 0.11 \text{ c}$ | 14.61 ± 0.85 a | $58.85 \pm 1.91 \text{ c}$ | 18.26 ± a |
| LSD≤0.05 | 0.365 | 1.377 | 4.683 | 1.316 |
| CV | 16.90 | 6.07 | 3.82 | 4.35 |

| Table 4. Effect of collective and individual effect of heat and drought stress on grain yield & chemical |
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| composition of wheat grain. |

Different small letters indicated that the means are significantly different ($p \le 0.05$), Values represent mean \pm SE (n =3)

| Table 5. Effect of individual and combined effect of heat and drought stress on chlorophyll contents, |
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| relative water contents, proline and soluble protein of wheat leaves. |

| | Chlorophyll contents | Proline (μmol g ⁻¹ FW) | Soluble protein (mg g ⁻¹ FW) |
|----------------|----------------------------|--------------------------------------|--|
| Control | 33.31 ± 0.93 a | 3.86 ± 0.46 c | $2.65\pm0.50~\text{b}$ |
| Drought | $27.90 \pm 1.07 \text{ b}$ | $8.23\pm0.85~b$ | $3.60 \pm 0.48 \text{ ab}$ |
| Heat | $26.47\pm0.50~b$ | $9.45\pm0.58~b$ | 4.34 ± 0.24 a |
| Drought + heat | 23.44 ± 1.66 c | 12.79 ± 0.43 a | 4.55 ± 0.86 a |
| LSD≤0.05 | 2.49 | 1.34 | 1.25 |
| CV | 4.76 | 8.32 | 17.74 |

Different small letters indicated that the means are significantly different ($p \le 0.05$), Values represent mean \pm SE (n =3)

Heat and drought stresses reduced the plant height, spike length, 1000 grains weight, spike length and grains yield, while increased gains proteins content. Our findings are highly in accordance with Kilic & Yagbasanlar (2010) where they argued that increased stress on wheat significantly reduced the wheat yield by influencing the negative impact on allometric and yield contributing traits as compared to control where no drought and heat stress was applied. Water shortage from seedling to maturity evidently lowered the grains yield more specifically grain numberfor each head (48%), amount of fertile ears for each unit area (60%), and dry matter weight. Hence stress usually depressed the grains yield of wheat (Hsiao, 1973) and can deteriorate the value of other yieldcontributingtraits of economic yield and enhanced the quality of gain protein (Guttieri et al., 2000).

According to results depicted in Tables 3 and 4, grains yield was greater in controlled soil (no water and heat stress) than in drought and heat alone and in combination might be due to low grains filling rate, grains filling duration and number of grains per spike. These results are in line with Kilic & Yağbasanlar (2010) who stated that wheat yield in well-watered plots were higher (4453-9550 kg ha⁻¹) while, under drought it reduced to 2208-3505 kg ha⁻¹. The higher yield in well watered environment might be due to higher gains filling period and more number of wheat grains per spike. Therefore, under stress condition the positive correlation was likely to observe among grains yield, grains filling rate, grains filling duration, plant height, grains for each spike, productive tillers number for each plant and spikelet numbers for each spike. These results were confirmed with Rashid et al., (2003) and Kilic & Yağbasanlar (2010) concluded that certain differences in relative yield values was observed with various wheat genotypes under water

stress condition. The number of spikelet per spike was decreased with applied drought and heat stress alone and in combination. This was in accordance with other researcher who stated that heat and drought stress stuckthe processes of reproduction that might be due to mainly ovule fertility, pollen fertility and negativity (Prasad *et al.*, 2011). Lower amount of grains per spike, reduced grains filling rate and weight was prominently reduced with sum of heat stress and drought. These consequences are according to Prasad *et al.*, (2011) and Mahrookashani *et al.*, (2017) concluded that drought and heat in combination decreased the number of grains for each foremost stalk of every cultivar with higher extent as compared to heat stress and drought individually.

Likewise length of spike, height of plant and grains number for each spike were directly link with grain yield of wheat. Correspondingly, Shams-ud-din (1987) observed that of grains numbers for each spike, spikes for each plant, harvest index, 1000 grain weightage, biological produce and glumes weight were directly proportional and associated with wheat grain yield. While, Simane *et al.*, (1993) used path analysis and stated that yield of grains and grains number per spike had positive relationship to grains yield of durum wheat under moisture stress and well water environment.

The phenome of more reduction in grains number for each spike was due to various reproductive processes. The drought stress inhibits development of flower pistillate and ovule function, whereas temperature stress reduced the pollen viability. Therefore, drought alone or with heat stress can enhance floret and spike concentrations of abscisic acid that might be associated with poor grain set (Yan *et al.*, 2012; Semenov *et al.*, 2014). Drought and heat can decrease photosynthesis, and the consequent sucrose dilution in the ear might be link with abortion of floret. Moreover, rise in temperatures (>30°C) in meiosis can hinder with division resultant to abnormal development of pollens (Semenov et al., 2014; Barnabas *et al.*, 2008).Heat stress reduced the wheat yield about 31% after 12 days of heading (Balla *et al.*, 2011). Rise in temperature in grain-filling stage had adverse effect on grains yield that may ascribed to severe decline in weight reduction (Tahir & Nakata, 2005) and lower in final starch content. The continuous rise in temperature may slow down or stops the starch synthesis as result of heat stress (Denyer *et al.*, 1994).

At high temperature, enzymes swiftly lose their activity, but sometimes they are capable of incomplete recovery when subjected to normal environment (no stress) (Jenner, 1994). Labuschagne *et al.*, (2009) stated that stress due to high temperature caused wheat weight loss results the lowered starch accumulation in wheat grains.

The influence of combined drought and heat stress reduced the grains yield and final starch accumulation and grains size, resultantly increased the protein contents in wheat grains, and the outcomes are according to Balla *et al.*, (2011) who said that introduction of combined drought and heat stress to wheat prominently reduced the wheat grains yield and starch accumulation, moreover significant increase in protein contents for all varieties (34.4% on average), that could be explained by reduced wheat grains size and weight. Whereas, only heat stress increased the protein contents about 10.5% and drought stress enhanced 23.2%, although this was not severe as in the combined drought+heat stressed wheat plants. Many scientists have also observed the enhancement of grains protein content in wheat due to high temperature stress (Bencze *et al.*, 2004).

The prominent increase of protein content in wheatgrains nonetheless was not linkwith enhanced grains quality as indicated by decline in gluten protein composition (Balla *et al.*, (2011). At extreme weather conditions, not only starch granule size, also protein components are also sensitive to stress (Zhao *et al.*, 2009).

Seed filling is important growth stage in all grains and take part in several biochemical progressions that associated to leaf assimilation and synthesis of carbohydrates, lipids, and proteins in the seeds. Plant biomass and seed yield both positively correlated with photosynthetic efficiency rates and chlorophyll content of plant leaves. This phenomenon suggesting that seed yield and plant biomass both were defined by impact of heat and drought application on photosynthetic capacity and assimilate transferal to the grain in applied stress periods (Awasthi *et al.*, 2017).

Our results highlight that more proline content was damage with combine exposure of drought and heat stress as compared with solely applied drought stress resultantly more reduction in grains filling rate and grains filling duration was observed under combined drought and heat stress. These results can be justify with Awasthi *et al.*, (2017) who concluded that oxidative damage in plants severely damage seed filling rate by hindering all metabolic developmental processes, thus disabling enzymes damaging membrane properties and degrading proteins contents that induce reduction in yield (Farooq *et al.*, 2009, 2016).

Conclusion

The current trial showed that influence of heat, drought and collective drought + heat stress prominently reduced the various growth and yield related physiological parameters. While, more reduction in wheat yield and growth was noted where combined stress (drought and heat) was applied. In contrast, grains protein, gluten and proline contents in flag leaf of wheat were increased under more stress environment (combined drought and heat). In future, more emphasis would be required to explore the influence individual heat, drought stress and their blend on stress tolerant and resistant varieties of various crops under field condition.

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