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Research Article

Understanding the responses of two wheat genotypes to individual and combined drought and heat stress during reproductive and grain filling stages

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Abstract

Drought and heat stress are the most devastating environmental stresses affecting wheat growth, development, and grain yield. Among all the phenological stages, reproductive and grain-filling stages are the most vulnerable stages results in significant yield loss. However, limited research has been conducted to examine the combined effects of both the stresses on wheat particularly at reproductive and grain filling stages. Therefore, the present study aimed to understand the response of wheat genotypes to individual and combined effect of drought and heat stress and to analyse the impact of such stresses on reproductive structures, yield and yield components. For this purpose, two genotypes (Gregory and Lincoln) were evaluated under control (CON), heat stress (HT), drought stress (DRT), and combined drought and heat (DRTHT) stress at the glasshouse facility at The University of Melbourne, Australia. Results revealed significant differences among treatments for floret fertility, pollen viability, pollen tube germination, chlorophyll content, leaf senescence, plant height, biological yield, grain yield and harvest index. Significant differences were also observed between genotypes for pollen viability, pollen tube germination, chlorophyll content and biological yield, whereas, non-significant for floret fertility, leaf senescence, plant height, yield and yield components. Overall, the stress treatments negatively affected all the traits under studied, where, combined effect of drought and heat stress was found to be more severe and intense on all the traits rather than their individual effects. Among genotypes, Gregory performed better than Lincoln for most of the traits under studied showing its tolerance to individual and combined effects of drought and heat stress. Identification of stress tolerant wheat genotypes may be useful for sustainable wheat production in areas prone to changing climatic conditions. Moreover, the stress tolerant genotype can be used in future breeding programs for variety development and genetic improvement of different wheat genotypes.

Keywords: Combined drought and heat stress; Drought stress; Grain filling stage; Heat stress; Reproductive stage; Wheat

Introduction

Wheat (*Triticum aestivum* L.) is the world's second most important staple crop, generally cultivated in dry and semi-dry regions, with an annual production of around 771 million tons [1]. However, by 2050, the world population is predicted to cross the 9.7 billion mark [2], with a projected global demand of double the current amount of food [3]. The major constraints affecting food production of a region are declining soil health, genetic diversity, increased incidence of biotic and abiotic stresses, or a combination of these, which are further aggravated due to climate change [4]. During the recent decades, research suggests that food security is already challenged by climate change due to changing temperatures, precipitation, and more frequent and erratic extreme events [5]. It is predicted that, due to changes in the climate, global temperature is expected to increase by 1.8-3.4 °C by the end of this century. This will result in more frequent and long-lasting extreme events such as heat waves and drought in the drier parts of the world including Australia [6].

Global wheat production is forecasted to decline by 6 % for every 1^{o} C rise in temperature [7] and more than half of the global wheat cultivation is affected by periodic drought (8,9). With changing climate, crops, including wheat, are encountering heat and drought stress individually and concurrently. The response of a plant to combined stresses might be additive or very unique [7, 10, 11] leading to differential adaptive genotypic responses due to triggering of interactive, inhibiting or differential prioritizing of signalling pathways [12]. This is further expressed phenotypically, leading to modified agro-morphological traits, plant growth, and development, which ultimately affect crop yield [12-14]. In order to develop crop varieties that can out-yield existing varieties amidst individual or combined effects of biotic and abiotic stresses, it is important to understand the genetic variation among wheat cultivars and their responses to such stresses (alone or combined).

Drought and heat stress influence all the phenological stages of wheat [15-18], but the reproductive and grain-filling stages are the most critical phases that result in major yield losses [19-22]. Drought stress during the reproductive stages prolongs the time of flower formation or even results in no flower formation [23], leading to less grains per plant and reduced yield. Wheat yield generally decreases by 1-30 percent if exposed to mild drought at the post-anthesis stage, but in the case of prolonged drought at anthesis and grain filling stages, yield can decline by 58-92 percent [24]. The optimum temperature required during wheat's reproductive and grain filling period ranges from 12 to 22^{0}C and a further rise in temperature substantially decreases grain yield [25-27]. High temperature during the anthesis stage leads to pollen infertility, slows the fertilisation rate, and shortens the grain filling duration [28]. Elevated temperature also results in accelerated wheat growth [29], due to limited time available to translocate assimilates for grain filling and thus declining the final grain yield [28].

Extensive research has been done to evaluate the individual effect of drought and heat stress on wheat during the reproductive and grain filling stages [22, 30-32]. However, not much has been researched to assess the combined effects of these stresses on crops, including wheat, particularly at the reproductive and grain filling stages [13, 16, 33]. Furthermore, the impact of these stresses on reproductive attributes and yield processes is still not well understood [33]. Barnabas *et al.* [34] highlighted the need for a comprehensive study to assess the combined effect of both these stresses on wheat under climate change regimes as the reoccurrence of combined drought and heat stress events will become more frequent in the near future.

In view of these research gaps, the present study aimed to examine a) the relative vegetative and reproductive growth performance and its effect on grain yield of wheat genotypes under drought, heat and combined stress and b) a comparison of wheat genotypes for their different levels of tolerance to individual and combined stress. Our hypothesis suggests that a) the wheat genotypes tolerant to drought and heat stress might not exhibit tolerance to the combination of these stresses, and b) the combination of stresses might have a specific response for one or the other trait for these wheat genotypes, which might not be observed under individual stress. This study will help us to understand better and unravel the specific and linked responses of wheat genotypes to individual (drought and heat) and combined stresses.

Materials and Methods

Plant materials and experimental location

The present experiment was conducted in a controlled environment glasshouse (22^0 C/14^0 C Day/night temperature) at The University of Melbourne, Parkville, Australia, in 2015. Two genotypes Gregory (Australian Premium White, 500mm rainfall zone, mid to late maturing) and Lincoln (Australian Hard, all rainfall zones, mid-maturing), were used in the present study [35]. The genotypes were sown in 25 cm diameter plastic pots in a completely randomized design with three replications. All the pots were filled with 10.50 kg of clay loam soil (6.4pH ($CaCl_2$), which was obtained from top 10 cm of cultivated wheat field of Dookie campus, the University of Melbourne. All the pots were irrigated before sowing to bring the soil to 100% field capacity [36]. Each genotype was raised in two pots per treatment per replication. Fifteen seeds of each genotype were sown manually at a depth of 2 cm in each pot. Plants were irrigated every two days to maintain the soil at 95-100% field capacity before applying stress conditions. Eight days after germination, the seedlings were thinned to seven plants per pot.

Treatment application

Four treatments were used in this experiment, control (CON), drought stress (DRT), heat stress (HT), and drought and heat stress combined (DRTHT). Based on Zadok's growth scale [37], drought stress (DRT and DRTHT treatments) was applied by completely withholding water when most of the heads started to show at the flowering stage (GS60-69). The drought-stressed plants of both treatments were rewatered after 25 days and continued to be irrigated until maturity. Heat stress (for HT and DRTHT treatments) was imposed by placing the pots in the growth chamber set at $34/22^oC$ day/night temperature with 14 hours day length for 5 days at the flowering stage [33]. In Heat stress (HT) treatment only, pots were watered regularly to maintain the soil moisture at 95-100% field capacity [36].

Data collection

Data was recorded on the following traits:

Pollen viability % was recorded on six randomly chosen florets from each genotype from each treatment during anthesis stage [38]. A total of 300 pollen grains per genotype per treatment were evaluated using compound microscope (model Leica DM 6000B) at 40X magnification power. The viable pollen was round and yellow in colour, while non-viable pollen was wrinkled and black in colour [39]. The pollen viability percentage was calculated by dividing the number of viable pollen grains by the total number of pollen grains [38].

Pollen tube germination % was determined by randomly selecting two spikes from each pot after approximately 24 hours of pollination. The spikes were fixed in 1:2 lacto-alcohol solution for 48 hours [40] and were transferred to 80% ethanol for long-term storage. Five styles, along with the stigma, were randomly selected and detached from each spike (total 10 styles) and assessed for pollen tube growth. A total of 300 pollen grains were observed from each genotype and treatment under compound microscope (model Leica DM 6000B) at 40X magnification power. The pollen grain was considered germinated when the pollen tube length was equal to half the diameter of the pollen grain [41, 42]. Pollen tube germination % was determined by dividing the number of pollen tubes germinated by the total number of pollens observed [43].

Floret fertility % was calculated as the ratio of total number of grains to the total number of florets per spike [38]. At maturity, all the plants were harvested (second pot for each genotype per treatment), and their florets were checked for grain by pressing between thumb and finger [33].

Leaf senescence % was recorded by counting the number of senescent leaves compared with the total number of leaves at time when each stress treatments imposition was stopped.

Chlorophyll content was recorded with SPAD 502 plus (Soil–Plant Analysis Development) device [44], when each treatment application was seized.

Data on yield and related traits were recorded at physiological maturity of wheat genotypes.

Data obtained was individually analysed using two-way analysis of variance in GenStat 16th edition, VSN International Ltd, Hemel Hempstead, UK [45]. Fixed model effects were treatments (CON, DRT, HT and DRTHT), and genotypes (Gregory and Lincoln), and replicates were used as a blocking factor. Multiple means comparisons were conducted using Duncan's test. Results were reported as means, and means were considered to differ significantly when $P \leq 0.05$.

Results

The results revealed significant effects of treatments on floret fertility, pollen viability, pollen tube germination, chlorophyll content, leaf senescence, plant height, biomass and yield and yield components. Whereas, significant differences were also observed between genotypes for pollen viability, pollen tube germination, chlorophyll content and biological yield, whereas, non-significant for floret fertility, leaf senescence, plant height and yield and yield components. Genotype and treatment interaction was found non-significant for all the traits under study.

Effect of different stress treatments on reproductive developments

The (Fig. 1) explains the main effects of genotype and treatment on floret fertility %, pollen viability % and pollen tube germination %. The bar chart regarding floret fertility illustrates that among all the treatments, both the genotypes exhibited their highest floret fertility under control conditions, followed by drought, heat and their combined stress. Gregory showed higher percentage of floret fertility than Lincoln in drought condition but lower percentage in combined effect of drought and heat stress. In case of pollen viability percentage, maximum pollen viability of 97% was recorded for control treatment, while, minimum viability percentage of 46% was recorded for combined effect of drought and heat. Between genotypes, highest mean value of 97% was observed for Gregory in control treatment than Lincoln with 96% of pollen viability. In drought condition, Gregory and Lincoln have pollen viability of 69% and 67%, respectively. In heat stress condition, Gregory showed greater pollen viability of 66% than Lincoln (61%). In combination of heat and drought stress condition, both the genotypes showed lowest viability percentage (Fig. 1). Viable pollen grains were found round and yellow, whereas, non-viable pollen grains were black and wrinkled (Fig. 2). A similar trend, with a significant reduction from control to both individal, and combined stresses, was also observed for pollen tube germination percentage. Between genotypes, pollen tube germination of Gregory and Lincoln were 79% and 77% respectively, in control condition. However, minor differences were observed between genotypes under drought stressed condition. In heat stress condition, Gregory showed 42% of pollen tube germination as compared to Lincoln (37%). The lowest pollen tube germination percentage was recorded in combined stresses. The (Fig. 3) showed pollen tube germination under different stress conditions.

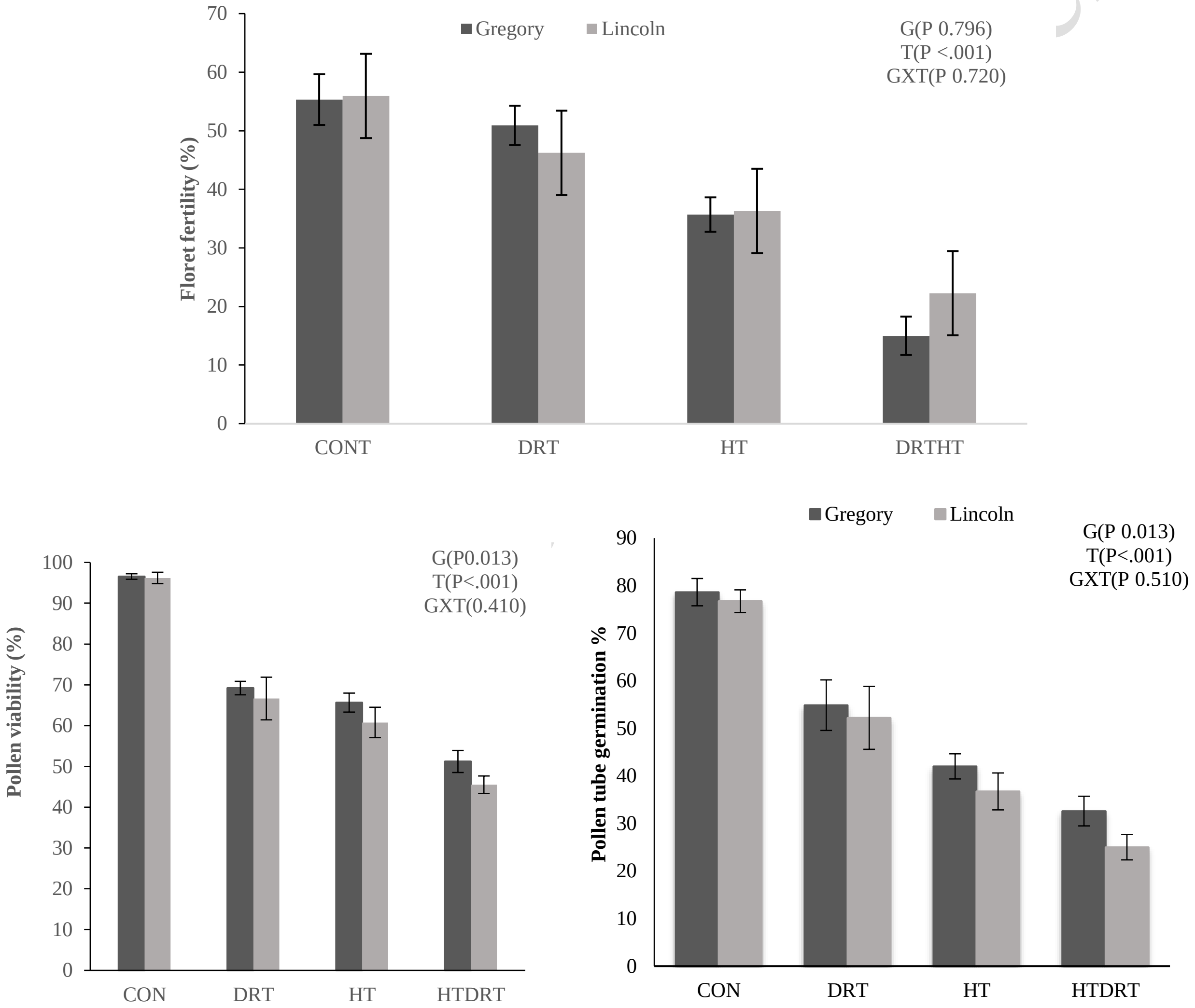

Figure 1: Effect of drought, heat and combined stress on floret fertility (%), pollen viability (%) and pollen tube germination (%) of Gregory and Lincoln wheat genotypes. Treatment as: CON=control; DRT=drought; HT= heat; DRTHT= combined drought and heat stress

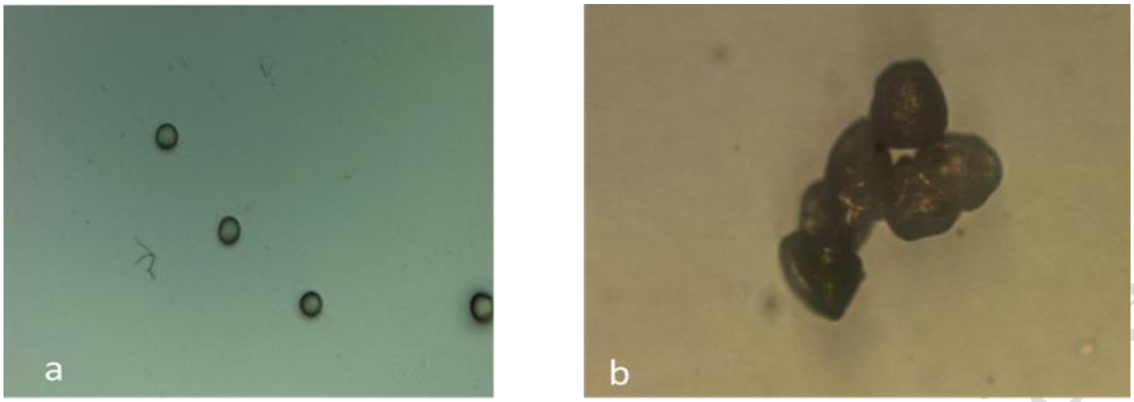

Figure 2: Pollen grain viability ascertained as a) viable pollen grains, which were round and yellow and b) non-viable pollen grains, which were black and wrinkled

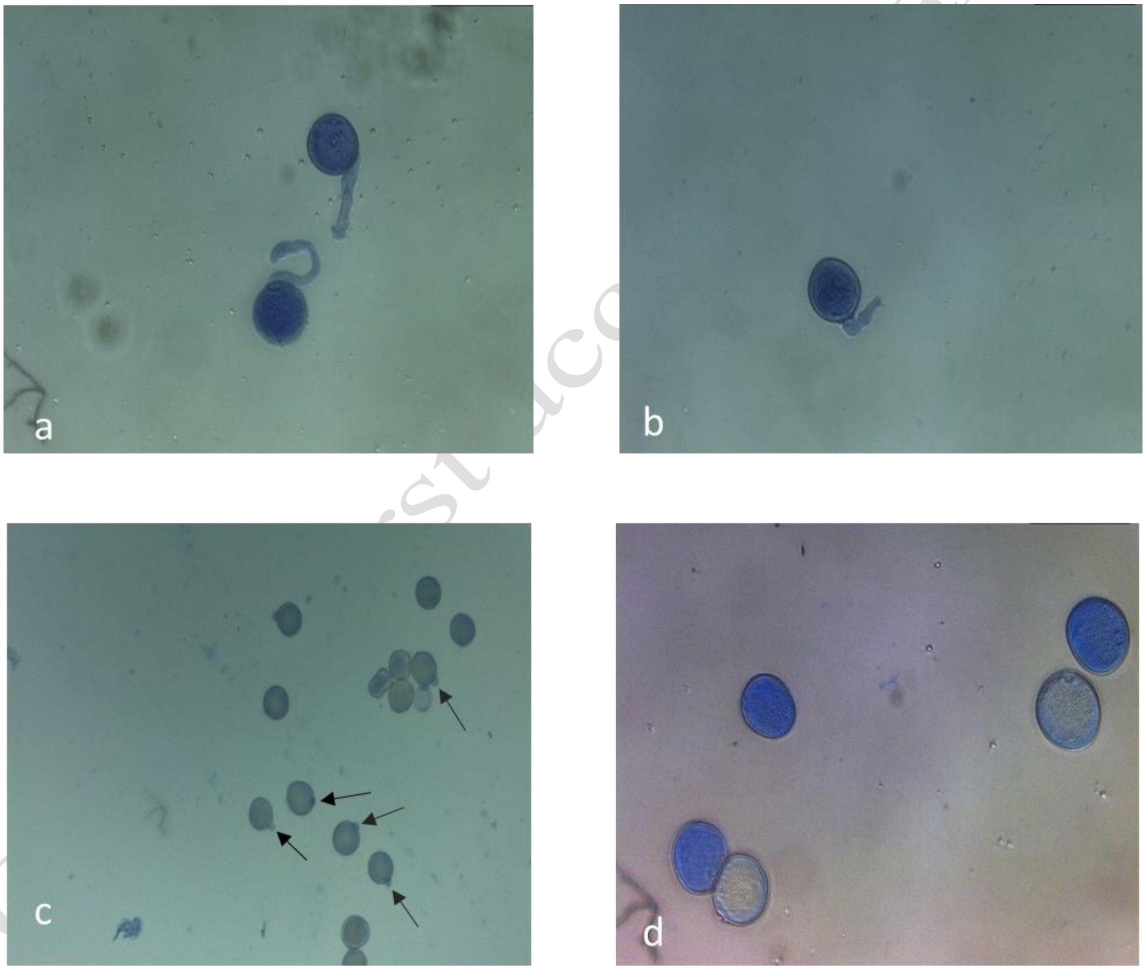

Figure 3: Decline in pollen tube germination of Gregory under a) control, b) drought, c) heat and d) combined drought and heat stress. The arrows on picture c show stunted pollen tube germination, whereas on d) there is no germination

Effect of different stress treatments on chlorophyll content and leaf senescence %

Both the genotypes showed higher chlorophyll content under the control treatment, followed by a significant reduction under DRT, HT and DRTHT (Fig. 4). The lowest chlorophyll content was recorded for the combined effect of drought and heat stress. Gregory showed higher chlorophyll content in all the stress treatments as compared with Lincoln. The percentage reduction in the chlorophyll content of Gregory was 34, 53 and 82%, whereas for Lincoln, it was 32, 67 and 88%, in response to drought, heat and a combination of drought and heat stress, respectively.

In case of leaf senescence, lower senescence value was observed in control condition, where both Gregory and Lincoln showed 14 and 12% of senescence, respectively. Whereas, in drought conditions, higher senescence of 35% was recorded for Lincoln as compared to Gregory with 20% senescence. The effect of heat stress was more severe on both genotypes (Gregory 53% and Lincoln 43%) compared to drought condition. The highest senescence value was noticed in combination of drought and heat stress conditions in both genotypes (Fig. 4).

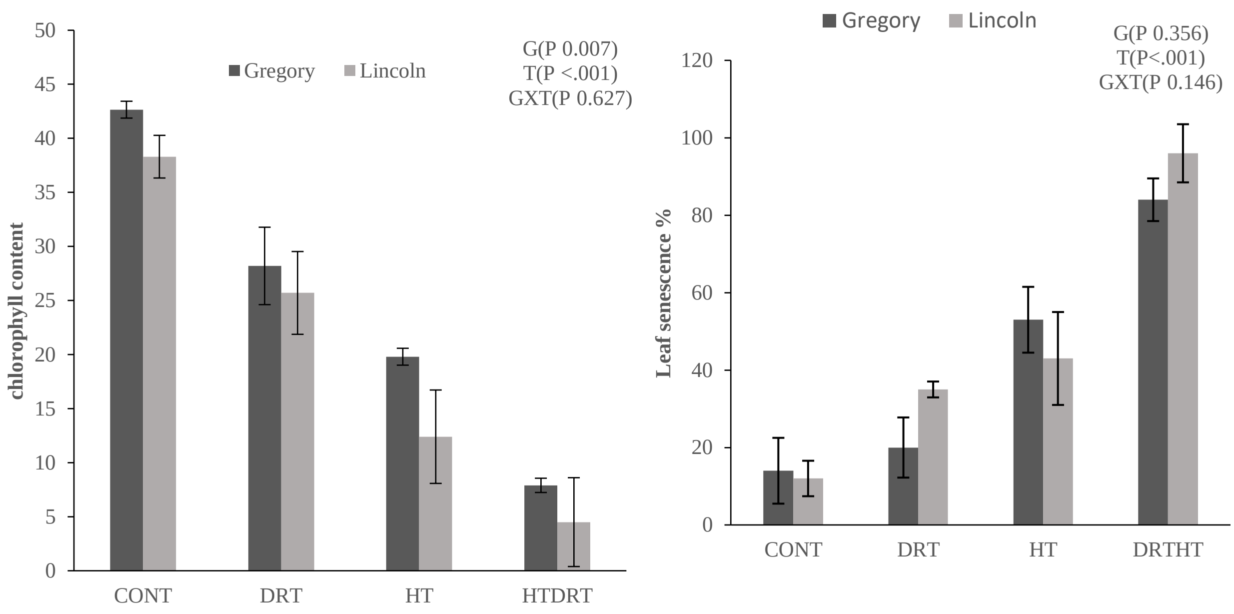

Figure 4: Effect of drought, heat and combined stress on chlorophyll content and leaf senescence % of Gregory and Lincoln wheat genotypes. Treatment as: CON=control; DRT=drought; HT= heat; DRTHT= combined drought and heat stress

Effect of different stress treatments on yield and related traits

From the (Fig. 5), it could be clearly seen that lowest spike length was recorded for combined effect of drought and heat stress as compared to other treatments. Between genotypes, spike length of Gregory is greater than Lincoln in all treatments. In drought condition, Gregory and Lincoln showed mean value of 4.96cm and 4.34cm, respectively. However, in heat stress, Gregory and Lincoln exhibited greater spike length of 5.77cm and 5.50cm, respectively. In combined effect of drought and heat, Gregory and Lincoln showed spike length of 4.48cm and 4.35cm, respectively. The main effects of treatments for the number of grains per spike declined under heat and combined stresses for both the genotypes. Gregory showed a significant decline and recorded the lowest number of grains per spike under combined stress. Plant height of the two genotypes was highest in the control and was significantly different from each other under the control conditions. A significant decline in plant height for Gregory occurred following drought, heat, and combined stresses. Whereas for Lincoln, there was a significant decline recorded only under the combined stress.

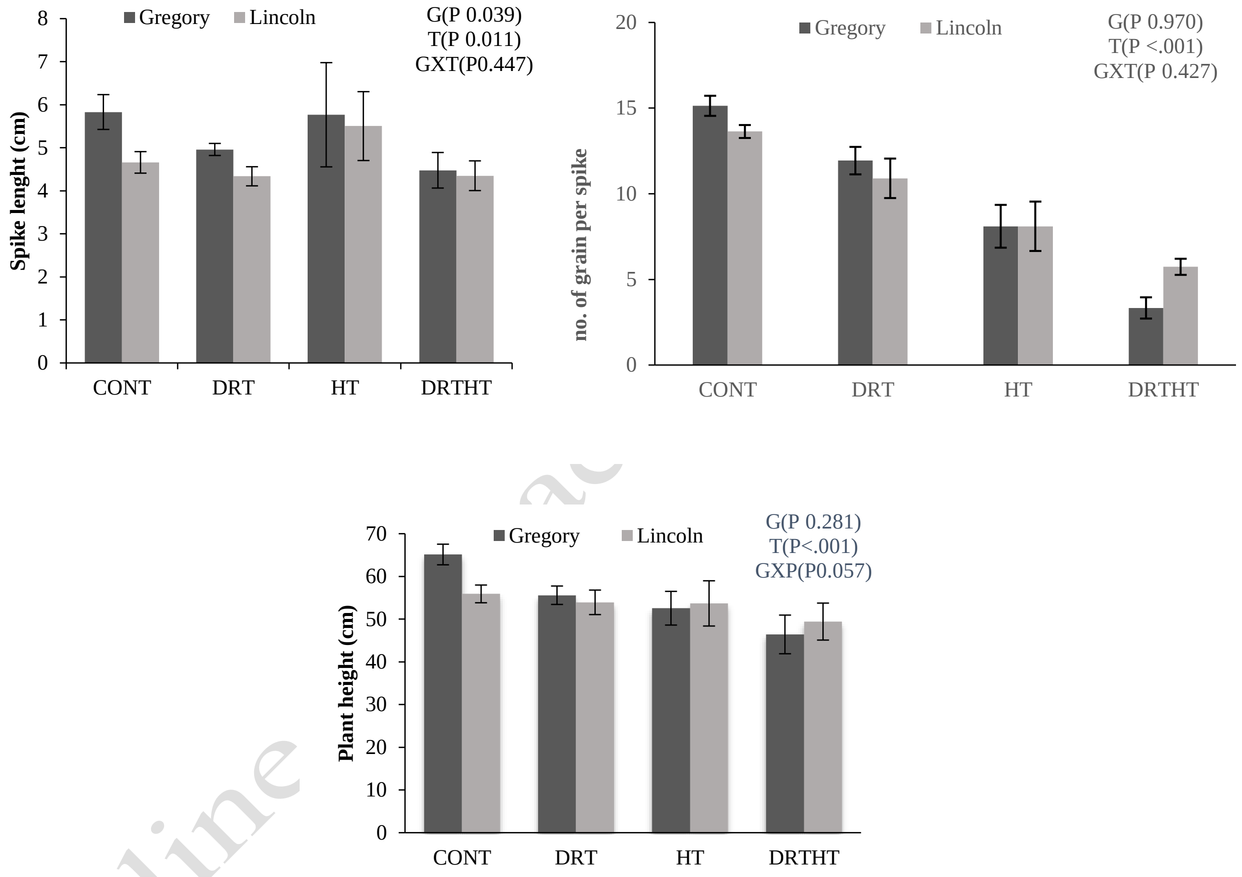

Figure 5: Effect of drought, heat and combined stress on spike length, number of grains per spike, and plant height of Gregory and Lincoln wheat genotypes. Treatment as: CON=control; DRT=drought; HT= heat; DRTHT= combined drought and heat stress

Gregory and Lincoln showed greater biological yield under control treatment followed by drought, heat and combined drought and heat stress (Fig. 6). In case of grain yield, maximum grain yield of 19.89g was obtained in control condition, whereas, minimum grain yield of 0.63g was recorded in combination of drought and heat stress condition. Between genotypes, maximum mean value of 19.89g was recorded for

Gregory, while, minimum mean value of 12.98g was recorded for Lincoln under control condition. In drought condition, maximum grain yield was again observed for Gregory (10.33g) than Lincoln (7.89g). Similar grain yield was obtained for both genotypes under heat stress. Overall, Gregory, showed higher yield under all treatments then Lincoln except for combined drought and stress treatment (Fig. 6). Regarding, harvest index, both genotypes showed a significant decline in harvest index from control, followed by drought, heat and combined drought and heat stress (Fig. 6).

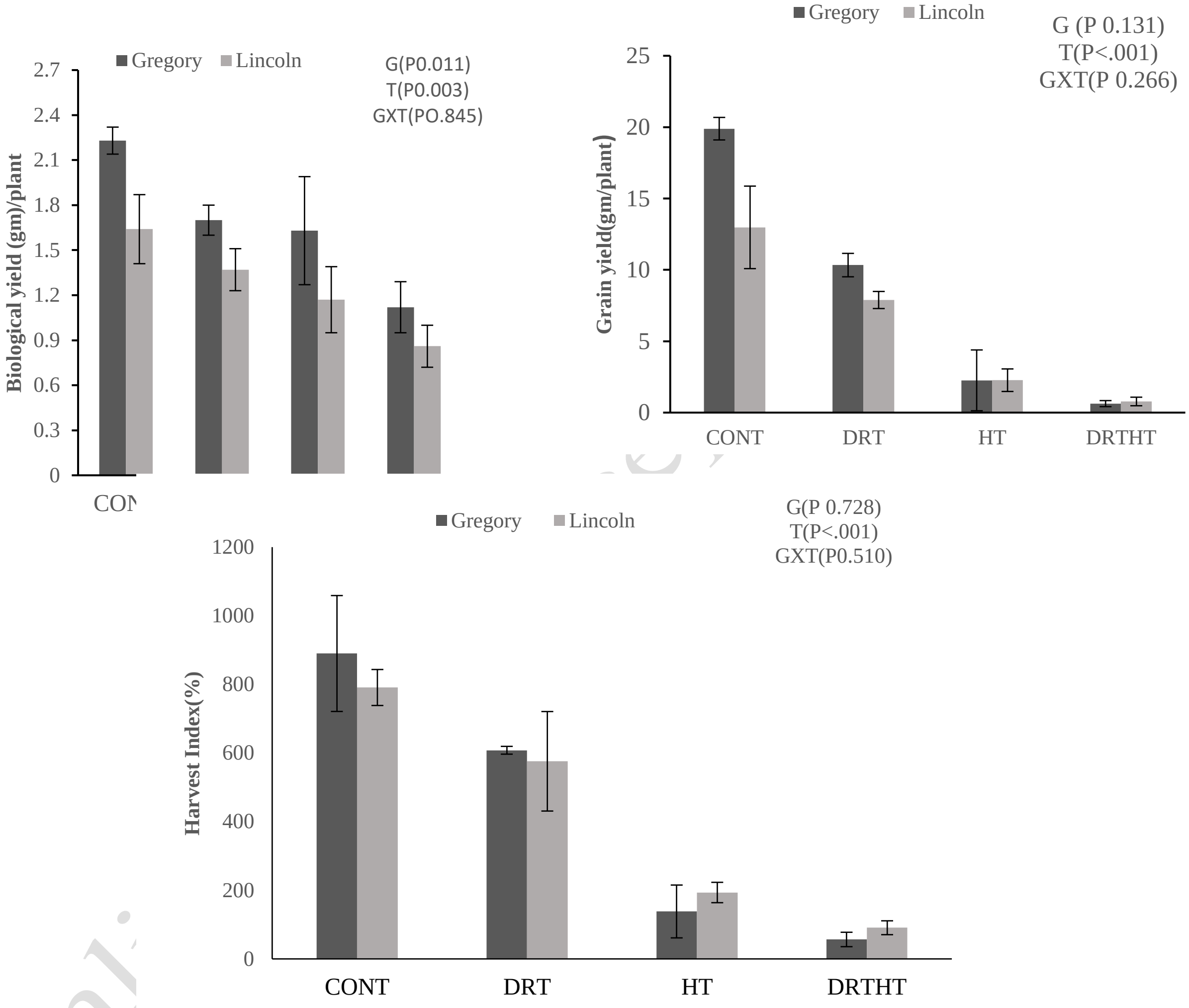

Figure 6: Effect of drought, heat and combined stress on biological and grain yield per plant and harvest index % of Gregory and Lincoln wheat genotypes. Treatment as: CON=control; DRT=drought; HT= heat; DRTHT= combined drought and heat stress

Discussion

To better understand the effect of individual and combined stresses on reproductive development and grain-filling stages, two selected genotypes (Gregory and Lincoln) were evaluated to unravel the genotypic differences, especially on floral fertility, which ultimately affects the final yield. The combined effect of drought and heat stress was more detrimental on floret fertility, pollen viability, pollen tube germination, chlorophyll content, leaf senescence, yield, and related traits than their individual effects. Past literature has also shown that the combined effect of drought and heat stress have a considerably greater damaging effect on crop growth, development and physiology than their individual effects **[46-49]**.

Successful fertilization mainly depends on the proper functioning of male and female reproductive organs including pollen viability, pollen tube germination and stigma receptivity. Therefore, failure of any of these functions significantly increases embryo abortion, leading to reduced numbers of grains and thus decreases the ultimate grain yield **[16]**. In this study, drought and heat stress significantly affected pollen viability and pollen tube germination, alone and in combination. Gregory showed greater pollen viability % and pollen tube germination than Lincoln in all the treatments. Similar effects have also been identified in other crops such as rice **[50]**, maize **[51]** and chickpea **[52]**. Heat stress affects the tapetal cells (nutritive cells) of the pollen grains and thus limits the distribution of nutrients in the developing pollen tubes, thus inducing pollen sterility **[53, 54]**. Moreover, drought stress also induces pollen sterility due to reduced availability of photosynthates in the flag leaf for the developing reproductive organs **[55]**. This might have led to a significant reduction in pollen viability of the two genotypes under drought stress and heat stress conditions. Based on the better performance of genotypes under individual stress it can be predicted that the combined stress would be more detrimental, which is clearly seen in the present experiment. This assumption is also supported by Grigorova *et al.* **[48]** and Sharma and Kaur **[49]**, who stated that the combined effect of drought and heat stress is more acute on the crop growth, development and physiology than their individual effects.

Any reduction in the chlorophyll content will lead to reduced photosynthesis and thus ultimately declines the final grain yield **[56]**. Gregory showed a higher chlorophyll content in all the stress treatments compared with Lincoln, which is consistent with Gregory having lower levels of leaf senescence. A significant reduction in chlorophyll content under stress conditions has previously been reported **[57-59]**. Furthermore, Prasad *et al.* **[33]** also examined the combined effect of drought and heat stress on chlorophyll content and found that the combined effect was more severe compared with the individual effects. The decrease in chlorophyll content under heat and drought stress is mainly associated with reduced stomatal conductance, reduced carbon dioxide fixation, and disturbed structure and function of chloroplast **[57, 60]**. This could be the possible reason why the chlorophyll content decreased under drought and heat stress.

Drought and heat stress deteriorate the chlorophyll content of leaves, therefore, accelerating the process of leaf senescence **[61]**. The increasing rate of leaf senescence reduces the available carbohydrate for the developing grain, leading to a significant decline in final grain yield **[62, 63]**. The present study observed increased leaf senescence under individual and combined drought and heat stress. Among the two genotypes, Gregory showed lower senescence in all treatments than Lincoln. Rivero *et al.* **[64]** showed that drought conditions accelerated the onset of leaf senescence, primarily due to cell death in the leaves and thus influenced the final grain yield.

Cereals are mainly cultivated for grain purposes, which is the product of several yield-related traits such as spike length, number of grains per spike and grain yield. In this experiment, significant differences were observed among treatments for spike length (cm), number of grains per spike and plant height. The lower number of grains is mainly because of induced pollen viability due to drought and heat stress **[65]**. Prasad *et al.* **[33]**, Kilic and Yağbasanlar **[58]** also reported significant effect of drought and heat stress on spike length, number of grains per spike and plant height in wheat genotypes. Similarly, significant differences were also observed between treatments for biological yield, grain yield and harvest index, where, combined effect of drought and heat stress produced the lowest biological yield, grain yield and harvest index. The low yield in this experiment might be due to reduced floret fertility leading to lower number of grains per spike. Guinata *et al.* **[66]** also reported significantly low yield among wheat genotypes in drought stress condition as compared to control. Moreover, Saeedipour and Moradi **[61]** also examined reduced grain yield, biomass and harvest index in drought stress condition as compared to well-watered condition.

Conclusion

Overall, the results of the present experiment revealed that both the genotype respond differently across different treatments and that the combined effect of drought and heat stress was more severe and acute than their individual effects. Gregory showed a higher pollen viability, pollen tube germination, chlorophyll content, spike length, and biological yield per plant than Lincoln in all the treatments. Gregory also exhibited a lower leaf senescence % than Lincoln under all stresses. Which showed tolerance of Gregory to individual and combined effects of drought and heat stress, indicating its resilience and potential for sustainable wheat production in regions projected to experience unfavourable climatic conditions. Moreover, stress treatments negatively affected all the traits under studied, where, combined effect of drought and heat stress was found to be more severe and intense on all the traits rather than their individual effects. This study has emphasized the need to better understand the impact of combined stresses on crop growth and final yield.

Author's contributions

Conceptualization, methodology, data collection, manuscript writing by: Lubna Naz, Software, data analysis, editing, referencing by: Sumayya Inayat.

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