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Research Article

Reduction in Morphological and Physiological Traits in Advanced Lines of Wheat (*Triticum aestivum* L.) under Normal and Lag Phase Heat Stress Settings

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ABSTRACT

Wheat is the staple food of many countries around the globe and approximately over three billion people depend on wheat for their daily calorie requirements. In several wheat-producing countries, wheat productivity has declined due to several biotic and abiotic stresses, largely due to heat and drought. The present study was designed to assess different morphological and physiological parameters in advanced lines of bread wheat under heat stress at the lag phase. Under heat stress conditions, the reduction in lag phase (19.7%) and relative water content (35.5%) was high in the wheat cultivar Subhani-21. Least decline in tiller plant^{-1} (16.5 %) and plant height (10.1 %) were perceived in advanced line HYT: 100-74 though the decrease in flag leaf area was 7.9 % in V: 20337. The smaller reduction in normalized vegetation index (29.32%) and chlorophyll content (10.62%) was detected in HYT: 100-47, indicating their stay-green trait while low canopy temperature values in heat stress as in HYT: 100-47 (-13.91%) and HYT: 100-74 (-12.9%) indicating their cooler canopies. The lowest value for % yield under stress conditions was observed in HYT: 100-47 (9.35%), followed by HYT: 100-76 (10.86%), HYT: 100-74 (11.33 %), and V: 21448 (14.19%) demonstrating their tolerance to heat stress. Canopy temperature was negatively correlated with all traits in the two states studied. These lines satisfy the standards of novelty, high yield, and heat stress tolerance. Therefore, these advanced lines could be valued and registered to combat heat stress in the country and sustain national food security.

Keywords: Responses, wheat, morphological, physiological, heat stress, lag phase.

INTRODUCTION

In Pakistan, the agriculture sector contributes 37.4 % to employment generation and 22.9 % to GDP and ensures national food security. Wheat is the backbone of food security and being the staple food of the people of Pakistan, provides basic nutritional requirements to the population. Wheat contributes 1.9 % to the national GDP and 8.2 % to value addition in agriculture (Finance Division, 2022-23). Punjab province is Pakistan's food basket where wheat planting is usually delayed when rice, cotton, sugarcane, and potato are rotated with it. Due to late sowing, wheat faces yield reduction due to low temperature in the early phase and higher temperature in the reproductive phase (Rehman *et al.*, 2009; Bana *et al.*, 2022). The late sowing of wheat is the major bottleneck of low average yield potential due to heat stress during the lag phase (from anthesis, grain filling to maturation) (Rehman *et al.*, 2009). During the lag phase, floral initiation, microspore development, and pollen tube expansion lower the grain filling duration, lower grain filling duration, rate, grain weight, grain, and grain number and shorten the life cycle than normal sown situation consequently resulting in yield reduction (Zulkiffal *et al.*, 2021).

It has been estimated that wheat yield significantly decreases with the increase of 1, 2, and 4 degrees above normal at the percentages of 17 %, 28 %, and 55 %, respectively due to effect on morphological, physiological, and biochemical pathways (Farhad *et al.*, 2023). Henceforth, our dynamic aim was to address heat stress at this phase in contrast with normal conditions by manipulating percentage reduction in morphological and physiological traits in advanced wheat lines.

MATERIAL AND METHODS

The field and tunnel experiments were carried out with 16 advanced lines with two checks (Arooj-22 and Subhani-21) at Wheat Research Institute, Faisalabad, Pakistan (73º74 E, 30º31.5 N, 184m). The area receives mean maximum and minimum temperatures of 27.73°C and 12.43°C, respectively during the wheat cropping season (November-April) while on an average basis, it became high (maximum 35.4 0C and minimum 21.55 0C) during the last fortnight of March and the first fortnight of April. During this period the crop is at the anthesis stage which is most sensitive to temperature stress. The high temperature during this period causes yield reduction due to adverse effects on morphological as well as physiological changes in wheat.

Trial treatments

To illustrate morphological and physiological traits, the field experiments were designed into two sets: normal and under a plastic tunnel in a plot dimension of 5.0 m x1.2 m rows using RCBD with two replications. The major criterion considered for the hybridization of these lines was their potential resistance to heat stress. Therefore, the tunnel was shielded with a plastic sheet from the lag phase till maturity for induction of heat stress. By recording daily temperatures inside and outside the tunnel, the temperature inside the tunnel was maintained above 38.6°C. (Figure 6).

Evaluation of Morphological traits

Seven morphological traits were recorded and analyzed. Plant height (PT) was measured from soil surface to tip of spike, spike length (SH) from the base to tip of spike using a scale, tiller plant-1 (TP), flag leaf area (LFA), lag-phase (LE) was calculated as number of days from anthesis, grain filling to maturation phases,1000 grain weight (GT) in gram and spikelet spike^{-1} (SE). For all traits, the average value was used from three randomly selected plants.

Evaluation of Physiological traits

Physiological heat stress indicators, viz., canopy temperature (Ce) at anthesis (0C), and normalized vegetation index (NI) at anthesis (0C) were measured with a handheld sense infrared thermometer (LT.300) and green seeker (505) respectively, in sunny day with slightest wind speed when the dew had desiccated from the plant canopy. Chlorophyll content and percent relative water content (RWC) were calculated using the formula = {(FT-DT) / (TT-DT)} x100.

Where, FT, DT, and TT are the fresh, dry, and turgid weights of leaves respectively. For FT, fully developed leaves from each advanced line were cut and then weighed immediately, for TT leaves were placed in a refrigerator (4 0C) for 24 hours by putting in test tubes filled with distilled water, then leaves were placed in blotting paper to absorb water from the surface and weighed. For DT, samples were finally placed in an oven for 48 hours at 80 0C and then weighed. (Barr and Weatherley, 1962). All routine cultural practices were equally exercised in two sets.

Statistical analysis

For future statistical analysis, Microsoft Excel 2016 was used to calculate the reduction percentages in the studied traits and STATISTICA version 5.0. was used for multivariate analysis to classify any credible arrangement among traits and advanced lines in two studied states (Sneath and Sokal, 2014).

RESULTS AND DISCUSSION

Deviation analysis

The deviation analysis at 1 % probability revealed the significant effect of advanced lines on eleven traits evaluated suggesting the advanced lines were genotypically divergent which makes it possible to select superior advanced lines in their respective states.

The heritability of a plant trait is considered very high when its value is equal to or over 80%. In this case, heritability for all traits is very high except LFA (moderate) and SLE (low). These broad sense heritability estimates (24-98 %) suggested the presence of ample scope of selection under both states individually and collectively. For LFA with moderate heritability suggested that selection should be delayed to more advanced generations while for SLE with low heritability implies that selection may be considerably difficult or virtually impractical due to the masking effect of the environment (Table 1). Our results are also in accordance with the results of Mahpara *et al.* (2018a) who also observed high heritability for SE, LE, PT, and TP.

Table 1. Combine variance components for morphological and physiological traits among wheat advanced lines under normal and heat stress states.

Variance components	PT	TP	LFA	LE	SLE	GT	SE	NI	CE	RWC	ChC
Heritability	0.85	0.92	0.46	0.94	0.24	0.87	0.94	0.85	0.74	0.89	0.89
Genotype variance	6.27	477.32	3.09	4.98	0.07	6.16	0.27	0.00	0.72	38.77	11.88
Residual variance	4.12	41.97	1.46	1.33	0.31	0.90	0.03	0.00	0.08	1.85	0.52
Grand mean	97.33	386.85	69.51	33.21	11.65	41.30	19.82	0.70	21.45	76.26	47.58
LSD	2.92	18.54	3.91	1.74	0.67	2.69	0.37	0.04	1.31	6.31	3.40

Whereas, LSD: Least significant difference, PT=Plant height, TP=tiller plant^{-1}, LFA=flag leaf area, LE=lag-phase, SLE=spike length, GT=1000 grain weight, SE=spikelet spike^{-1}, NI=normalized vegetation index, Ce=canopy temperature, RWC=relative water contents, ChC=Chlorophyll content

Reduction in morphological traits

Heat stress forces the plant to enter the reproductive stage earlier than normal which consequently results in reduced tillers plant^{-1}, plant height, and lag period (Rehman *et al.*, 2009). The reduction in lag phase occasioned grain weight ultimately grain yield due to spore abortion, which stemmed activation of starch metabolism in the kernels. Grain filling duration determines grain development which further improves the grain weight. That is why the reduction in lag phase in heat stress conditions was high in Subhani-21 (19.7 %) and V: 21339 (18.0 %) concerning the lowest reduction in HYT: 100-47 (9.0 %) and HYT: 100-74 (9.5%). Under heat stress this lag phase reduction demonstrated high grain weight reduction in Subhani-21 (26.1 %), and V: 21339 (21.4%) in contrast to HYT: 100-47 (8.8 %) and HYT: 100-74 (8.9 %). Herrera and Calderini (2020) also validated that heat shocks during the lag phase might affect the final individual grain weight (16 %) mainly due to the reduction of rate and duration of this period. Therefore, the high grain-filling period, frequency, and high latent grain weight should be used as a selection criterion to combat heat stress in wheat. Similarly, a maximum reduction of tillers and plant height was observed in Subhani-21 (23.5% and 14.9 %) while the minimum decline of 16.5 %, 10.1 % in HYT: 100-74, was perceived under heat stress conditions for the same traits. The discount in leaf area arrays was from 7.9 to 19.2 % in V: 20337 and V: 21448, respectively. Under stressed conditions, the high temperatures has a detrimental influence on wheat development that helps to mature earlier than normal producing reduced leaves, tillers and plant height which ultimately and ramblingly distress the final yield. Another study suggested (Gao *et al.,* 2017) that improved growth rate in wheat is driven by increased accumulation of water-soluble carbohydrates. That is why under heat stress condition accumulation of water-soluble carbohydrates will decrease that resulted in reduction of leaves, tillers and plant height. Contradictory results about plant height lessening and upsurge genetic gain were found in which breeding novel wheat genotypes with dwarfing genes significantly contributed to increased wheat productivity globally (Zhang *et al.*, 2016). This contradiction may be because in our study plant height reduction is due to the environmental factor (heat stress) but not due to genetic factors.

Under stress conditions maximum reduction in spike length, grains spike$^{-1,}$ and 1000 grain weight were observed in subhani-21(9.3 %), (4.4 %) (26.1 %), respectively while the minimum reduction in these traits were 2.5 %, 0.7 % and 8.8 % in V: 7016S, V: 20337 and HYT: 100-47, respectively. The reduced number of grains per spike might be due to low grain fertility associated with increased temperature while the possible reason for the decrease in grain weight trait might be attributed to reduced duration lag phases. Spike length, grains per spike, and thousand-grain weight were reduced more under late sowing which faces terminal heat stress was also reported by Rehman *et al.* (2021). The lag phase reduction was noted to be maximum in Subhani-21 (18.7 %), which was at par to V: 21239 (18.2%) while these values were in minimum at the par range for HYT: 100-74 (9.5 %) and HYT: 100-47 (9.4 %). During the lag phase heat stress air and soil temperature reach a threshold level which shortens the reproductive phase and the strength of nutrient translocation from sink to source becomes weakened. The shortening of this phase due to elevated heat stress resulted in low grain weight. Zulkiffal *et al*. (2022) reached the same results while evaluating the stability of heat-stress tolerant and susceptible wheat lines in eight varied sowing environments (Figure 1).

Reduction in physiological traits

Normalized vegetation index (NI) determined through sense infrared thermometer and ChC by SPAD meter are indirect and direct indicators of chlorophyll content. Wheat production under heat stress can be improved by selecting wheat lines with a high NI and ChC value. The less reduction in NI and ChC was observed in HYT: 100-47 (29.32, 10.62) indicating their stay-green character retaining and maintaining the ChC during high-temperature stress that may depend on the lines' genetic component.

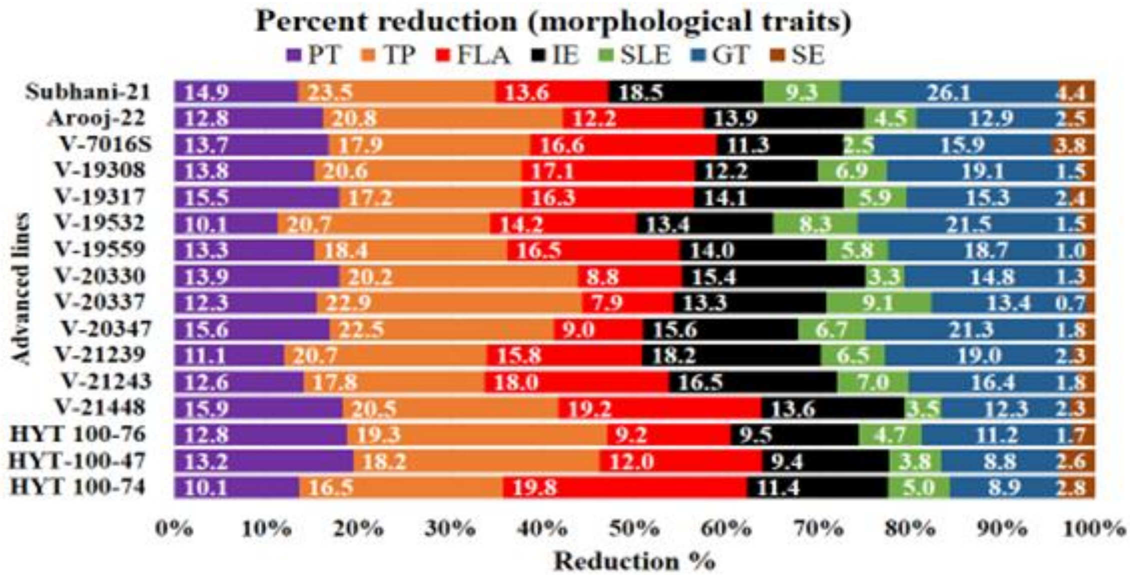

Figure 1. Estimation of percent reduction in morphological traits in wheat advanced lines, whereas, PT=Plant height, TP=tiller plant^{-1}, FLA=flag leaf area, LE=lag-phase, SLE=spike length, GT=1000 grain weight, SE=spikelet spike^{-1}.

The most frequent reduction of NI and ChC was perceived in Subhani-21 (8.46, 4.14). Kaur and Thind (2017) studied genotypes and publicized that chlorophyll content decreased with increased temperature stress. High reduction of NI and ChC indicated loss of photosynthetic pigments, and high photosynthesis disorder by reducing photo inhibition high sensitivity of chloroplast thylakoid membranes which finally prompts leaf senescence under vulnerable heat stress.

Low CE values in heat stress as in HYT: 100-47 (-13.91) and HYT: 100-74 (-12.9) indicate their cooler canopy which increases the rate and duration of the lag period which eventually decreases the %YD than high CE values lines Subhani-21, V: 7016S and V: 20330 (-8.08 to -8.92). RWG provides a water deficit level idea and is the best pointer to estimate dryness and aridity resistance. A wide range reduction under stress conditions was observed in RWC from 14.3 % (HYT: 100-47) to 35.5 % (Subhani-21). Zulkiffal *et al*. (2021) reported a 26% reduction in RWC under heat stress in wheat. As an indicator of water status, relative water content is the meaningful determinant of heat tolerance because it signifies the membrane stability and balance between water supply and evapotranspiration. Therefore Subhani-21 with low relative water content retention has high transpiration and stomatal conductance may be due to shorter root penetration and short water use efficiency.

Overall from this physiology study it is exposed that under heat stress, the vicissitudes in NI and CE efficiently signified vicissitudes in RWG and %YR in such a way that the lines that uphold high NDVI, RRWC, and low CT at lag phase could be painstaking a measure of heat tolerance (Figure 2). Due to heat stress, the physiological and morphological traits of wheat are interrelated and affected leading to a decline in growth and yield. Physiological replies to heat stress comprise reduction of grain filling period, reduced photosynthesis, early leaf senescence, and changed plant and water interactions (Waheed *et al.,* 2021) while in morphological traits reduction in plant height, leaf area, productive tiller, grain size, and weight (Zulkiffal e*t al*., 2021).

Percent yield reduction (%YR)

The bottommost value for % YD under stress conditions exposed to HYT: 100-47 (9.35 %) lagged by HYT: 100-76 (10.86%), HYT: 100-74 (11.33 %), V: 21448 (14.19%) and the uppermost reading noticeable to Subhani-21 (41.8%) trailed by V: 19532 (30.40%) and V: 20347 (29.36%). On the basis of higher values for %YR these lines can withstand heat stress and are heat tolerant. The at par % %YR (21-23 %) was found in lines V: 7016 S, V: 20337, V: 19559, and V:19517 which showed the same effect under heat stress conditions (Figure 3).

Djanaguiraman (2020) also signposted that during the anthesis and grain filling stages wheat yield significantly decreased by 29 and 44%, respectively due to high temperature. The simulated consequences disclosed that terminal heat stress will lessen wheat yield by 11.1%, by 2050 and adaptation of appropriate approaches will help in reducing the influence of heat stress to 9% by 2050 heat stressed consequences (Dubey *et al*., 2020).

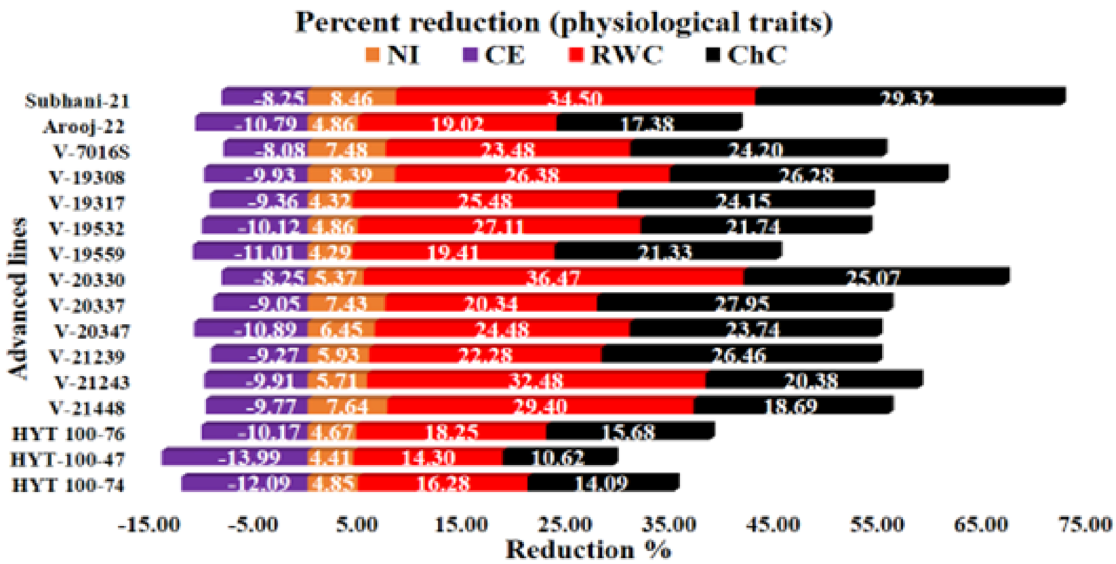

Figure 2. Appraisal of percent reduction in physiological traits in wheat advanced lines. Whereas, NI=normalized vegetation index, CE=canopy temperature, RWC=relative water contents, ChC=Chlorophyll content.

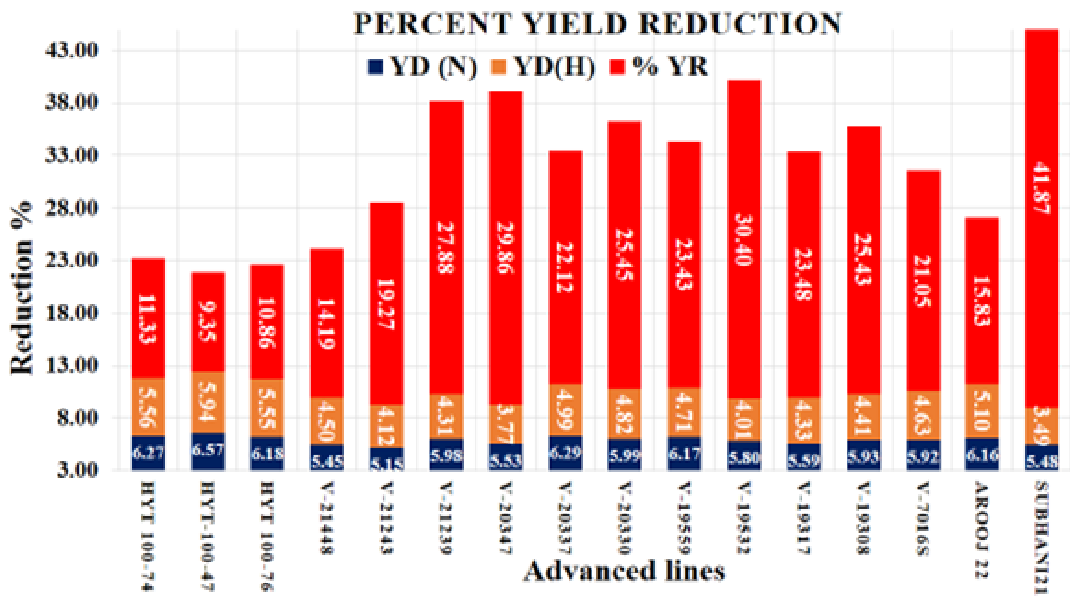

Figure 3. Estimation of percent yield reduction in wheat advanced lines. Whereas, YD (N) =Yield under normal, YD (H) =Yield under heat stress, %YR=Percent yield reduction.

Multivariate analysis

Correlation analysis

Knowledge of the relationship among wheat traits is valuable while probing these traits for heat resilience. The correlation among morphological and physiological attributes evaluated in this study is shown in Figure 4. Among physiological attributes, results demonstrated that CE displayed a negative association with all traits except LE under both states. This negative incline in CE advocated that genotypes with cooler canopies display higher GT and yield because cooler canopies recover physiological functions by increasing the duration and rate of the grain filling mainly under a heat stress state. The negative association of CE with NI is because as the lag period progressed, NI values in genotypes decreased with the increase of CE value. Such reprisals to canopy cooling have been affirmed by Pecetti and Damania (1994). ChC has a strong positive correlation with NI and RWC in both states. The ChC and RWC data provide information on NI. Progressive ChC and RWC values indicate positive association with NI and vice versa which suggested the presence of stay-green traits in heat stress state. A constructive link of NI and ChC is also recommended because the lines with high ChC during the lag stage exhibit higher grain yield. Consequently, both traits could be considered as an operative measure for developing heat-tolerant varieties (Wang *et al.*, 2022). GT was found to be positively correlated with all physiological traits in the two states studied except CE. In morphological attributes, GT has a positive correlation with all traits except LE in two states. Increased GT weight directly contributed to enhanced yield due to higher grain filling rate and longer active filling period. In the stress state, the grain filling period remained short due to forced heat maturity which did not permit to increase in the GT. Song *et al*. (2015) also reported that the rate of grain filling was smaller than the duration of grain filling at elevated temperatures which eventually decreased the grain weight and yield. The reduction in GT (26.1, 8.9 %) and %YR (41.78, 11.3 %) were more in heat susceptible than heat tolerant lines, respectively. Modarresi *et al.* (2010) formerly pronounced that elevated temperature significantly decreased 1000-grain weight (20.61%) and grain yield (46.63%) under heat-stress conditions.

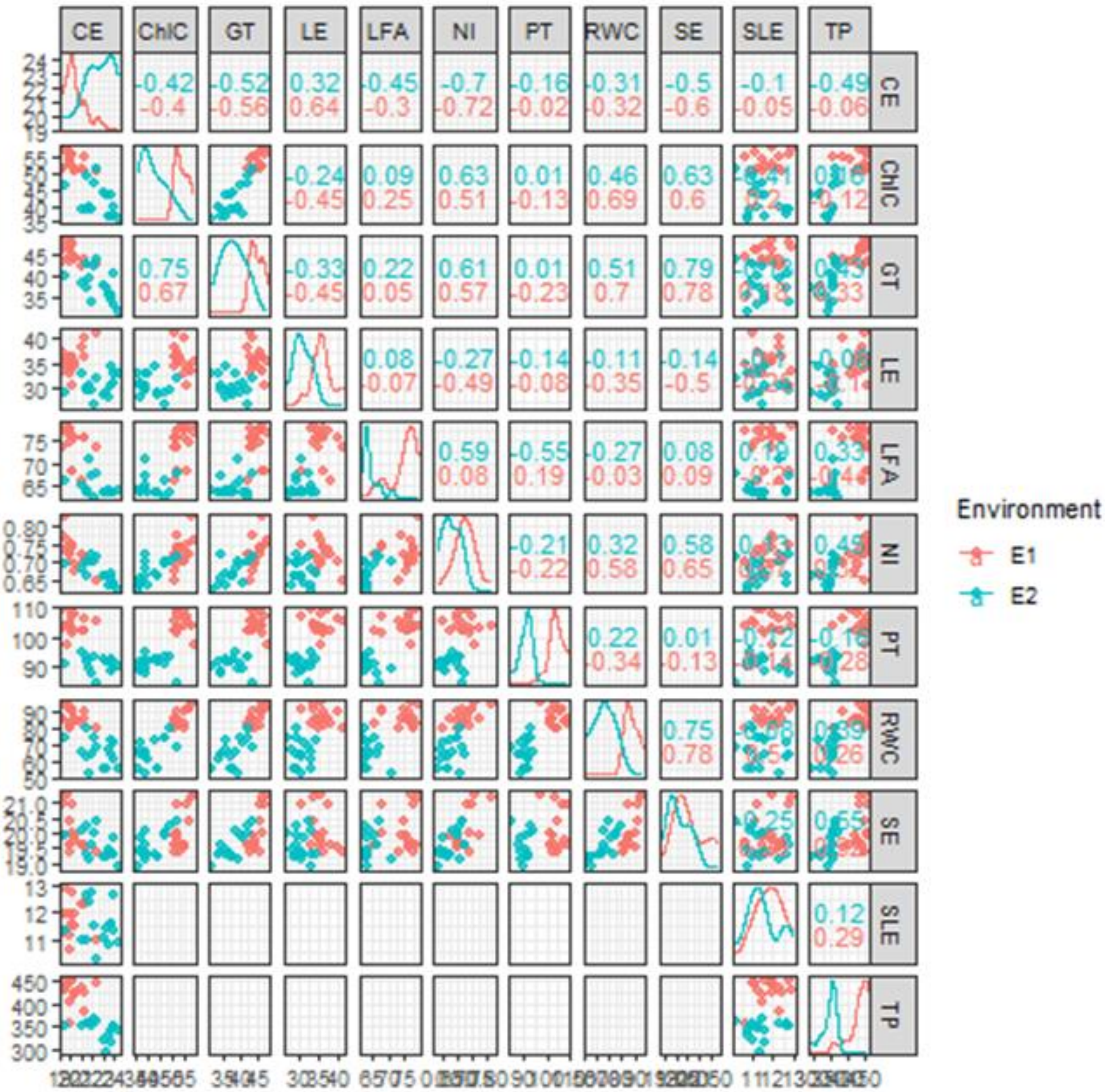

Figure 4. Acquaintance of morphological and physiological traits under normal and heat stress states. Whereas, Ce=canopy temperature, ChC=Chlorophyll content, GT=1000 grain weight, LE=lag-phase, FLA=flag leaf area, NI=normalized vegetation index, PT=Plant height, RWC=relative water contents, SE=spikelet spike^{-1}, SLE=spike length, TP=tiller plant^{-1}.

Principal component analysis

Principal component analysis abridges bulky data tables. The first two PC elucidate concentrated unevenness. Overall, 76.4% of the evidence limited in the figures were booked by the first two PCs. The first PC accounted for 64.3% of the total inconsistency of the data whereas the second PC explains 12.1% of the total variability (Figure 5). Consequently, we might want to halt at the second principal component. Regarding advanced lines by traits ranks. The distance between the advanced lines and the biplot basis measures the advanced lines individuality in such a way that advanced lines with longer distance exerted strong interactive forces in that particular state and vice versa. For instance, HYT: 100-76 trailed by HYT: 100-79, V: 20347, HYT: 100-47 and V: 20337 were located far from origin, reflecting major influence in heat stress state. The advanced lines Subhani: 21, V: 19559, V: 21243 and V: 19532 were adopted to normal state while V: 19308 showed similar interaction in both states. Figure 3 also delivers indication that % YD under stress condition were less in heat adopted lines and vice versa. Regarding traits evaluation, the projection of traits on PCs revealed that LE, PT and LFA had positive correlation in normal state while SLE, SI and NI had positive correlation in heat stress state (acute angles). CE negatively correlated (obtuse angles) with yield in whole two states (obtuse angles). Our findings are exactly in accordance with Shah *et al*. (2022) recently establish negative associated expression of CE with all other morphological traits.

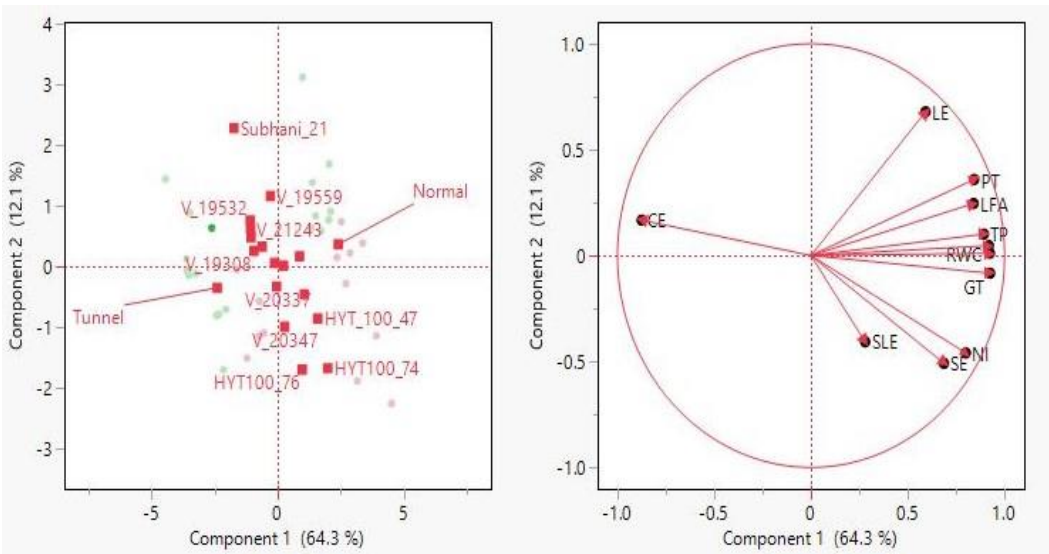

Figure 5. Bi-plot for traits and advanced lines.

Figure 6. Establishment of heat stress and normal states in the tunnel and field.

Figure 2 also provides evidence that CE negatively correlates with all other morphological traits which is why there are low CE values in heat stress adopted lines indicating their cooler canopy which increases the rate and duration of the lag period which eventually decreases the %YD than high CE values advanced lines. The length of the trait vectors is directly proportionate to the standard deviation and describes the discriminating ability of the traits inside the state.

Hence, in heat stress SE, NI, and, in normal LE and PT were utmost perceptive (informative) while SLE in heat and LFA in normal conditions were the slightest discriminating.

CONCLUSION

In the view of seven morphological and four physiological traits reduction under heat stress and normal states, the bottommost value for % YD under stress condition exposed to HYT: 100-47 (9.35 %) lagged by HYT: 100-76 (10.86%), HYT: 100-74 (11.33 %), V: 21448 (14.19%) and the uppermost reading noticeable to Subhani-21 (41.8%) trailed by V: 19532 (30.40%) and V: 20347 (29.36%). On the basis of lower values for %YR these lines can withstand heat stress and are heat stress tolerant. The at par % YR (21-23 %) was found in lines V: 7016 S, V: 20337, V: 19559, and V: 19517 which showed almost the same effect under heat stress conditions. Therefore, these could be valued, close-fitting, and registered for combating the heat resilient scenario prevailing against national food security.

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AUTHOR CONTRIBUTIONS

MZ mainly conducted the research, AA and JA helped in data analysis, MO, MHT and SA assisted in data recording and MA, MIK, IG, AK and AJ helped in manuscript writing and reviewing.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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