

Physiological selection of wheat synthetic back derived lines for drought and heat tolerance

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Abstract

Drought and heat stress are major constraints to wheat production, necessitating the identification of genotypes with superior physiological mechanisms for stress adaptation. The present study evaluated 200 synthetic backcross-derived lines (SBLs) for drought and heat tolerance using germination, physiological, and yield-related traits. For drought screening at the germination stage, seeds were exposed to 20% polyethylene glycol (PEG-6000), and germination percentage, shoot length, and root length were recorded. Significant variation was observed among SBLs, with germination ranging from 0 to 100%. Forty-seven SBLs exhibiting high germination also showed greater seedling biomass. Greenhouse evaluation of 193 SBLs and the check variety NARC-2009 under drought stress identified 31 SBLs as drought tolerant based on grain weight per spike, thousand-kernel weight, and relative water content (RWC). These lines exhibited only 10–20% relative yield reduction compared with 25–50% in the remaining lines. Heatmap and correlation analyses demonstrated positive associations of RWC and membrane

stability index (MSI) with thousand-kernel weight, indicating their potential as reliable physiological indicators of drought tolerance. For heat tolerance assessment, plants were exposed to 40 °C for 5 h daily at the post-anthesis (milky) stage. Based on yield and physiological responses, 45 SBLs showed minimum reductions in grain weight under heat stress. These lines maintained higher grain weight per spike and thousand-kernel weight and exhibited greater proline accumulation. Correlation analysis further revealed a positive association between proline content and thousand-kernel weight under heat stress. Conclusively, the study identified promising SBLs with superior drought and heat tolerance and demonstrated that RWC, MSI, and proline accumulation are useful physiological traits for screening wheat germplasm. The selected lines may serve as valuable genetic resources for wheat breeding programs aimed at developing climate-resilient cultivars with improved productivity under drought and heat stress. © 2026 The Author(s)

Keywords: Drought stress, Heat stress, Proline, Relative water contents, Synthetic back derived lines

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Introduction

Wheat is the staple food for 35% of the world's population and meets 55% of carbohydrate, 20% of calorie and protein needs of human diet (Iqbal et al., 2018; Mehmood et al., 2020; Dinsa & Balcha, 2024; Sharma & Sharma, 2025). Pakistan, despite being among the world's top ten wheat-producing countries, continues to face significant food insecurity, with approximately 22% of its population being undernourished (Abbas & Shafique, 2019; Alamgeer et al., 2022). The country is also ranked 11th among the world's hunger- and malnutrition-affected nations. The country is also prone to climatic changes (drought and heat) and alarming rise of population which is undermining the crop yield (Mustafa et al., 2021; Habib-ur-Rahman et al., 2022).

According to an international report, Pakistan remains among the countries most vulnerable to climate change. According to the Climate Risk Index 2025, Pakistan was ranked as the most affected country globally for the year 2022, largely due to the catastrophic floods that affected more than 33 million people and caused economic damages and losses exceeding US\$30 billion. The agriculture, food, livestock, and fisheries sectors were among the most severely affected, with estimated damages of approximately US\$3.7 billion (International Monetary Fund, 2025).

Primary synthetics of wheat are poor in terms of land management and crop production, specifically tall, poor qualitatively, difficult in threshing, having low or poor yield, but still, they have useful variations for the scope of economically important distinguishing features (Khan et al.,

2021). From 1991 onward, almost 186 winter habits and 1014 spring habit primary synthetics have been brought forth by International Maize and Wheat Improvement Center (CIMMYT). About one third of the advanced lines that CIMMYT has widely distributed to world breeding programs for both irrigated and rainfed regions are synthetic derivatives. In Spain, a synthetic derivative was certified by the name “Carmona” because of its higher commercial potency, reaching maturity and primordial phenology (van Ginkel & Ogonnaya, 2007).

Drought and heat are the main abiotic stresses affecting wheat yield. Approximately 33% of the Pakistan’s arable land area is rain-fed where a transient drought is a common phenomenon. Likewise, late sowing of wheat in the country after the optimum sowing dates, mainly due to late harvesting of cotton, rice and sugarcane expose the plants to terminal heat stress (Raza et al., 2019). Planting after a deadline of 20th November causes a 1 kg/acre/day reduction in yield (Muneer et al., 2025). Wheat varieties grown nowadays have a narrow genetic base because of consistent inbreeding. There is a need of wider wheat genetic resources for incorporation into our existing wheat varieties. Synthetic hexaploid wheat (SHW) utilizing A, B and D genomes of wheat wild relatives hybridized with *Aegilops tauschii* L. have greater D genome diversity and greater ability to resist biotic and abiotic stresses (Li et al., 2014).

Optimum soil moisture is a lifeline for successful wheat production but in Pakistan wheat is mostly sown on residual soil moisture after October rains which on one hand poses a temporary drought situation and on the other hand exerts extra pressure on plant metabolic processes in forced deep rooting in order to tap soil moisture from lower soil horizons (Jiskani, 2001; Asif et al., 2019). Plant yield is an intricate balance between individual yield related physiological phenomena like source-sink relationship, photosynthetic carbon assimilation and its utilization in plant metabolism. All these complexities have recently regenerated a new branch of plant science “physiological breeding” that entails a detailed study of yield components at micro level followed by identification of elite lines suitable for incorporation into future breeding programs (Rynolds et al., 2012; Sales et al., 2022; Kujur et al., 2024).

Zinc and Fe deficiencies in human food badly affect a large proportion of the world's population. This is either due to deficiency in soils or inefficiency of crop plants to absorb these nutrients from soil (Imtiaz et al., 2010). Zinc deficiency can lead to stunted growth, diarrhea, and pneumonia in children, with the latter two conditions contributing substantially to infant mortality, whereas iron deficiency is a major cause of anemia (Escobedo Monge et al., 2019). Nearly 500,000 children under 5 years of age die annually because of Zn and Fe deficiencies. In Pakistan, the incidence of micronutrient malnutrition particularly zinc & iron deficiencies is also very high (Hwalla et al., 2017). In this regard strategies and activities have been initiated for biofortification of iron in

important cereal crops under HarvestPlus Program across ten Consultative Group for International Agricultural Research (CGIAR) member countries of the world (HarvestPlus, 2018). It has been observed that modern bread wheat has much lower concentration of iron and zinc in their grain as compared to wild tetraploids and diploid wheat. CIMMYT-bred 1014 synthetic hexaploid wheat (SHW) have been tested in Pakistan for abiotic and biotic stresses and 260 lines have been found as resistant sources against heat, drought, salinity and waterlogging, and spot blotch, karnal bunt, Septoria leaf blotch and Fusarium head scab stresses (Mujeeb-Kazi et al., 2008; Tiwari, 2021). These primary synthetics though have a varying degree of drought tolerance, do not necessarily express improved yield under drought stress when compared to modern wheat cultivars (Reynolds et al., 2012). So, a number of these SHWs have already been crossed to elite wheat cultivars across the world and the resultant germplasm termed as synthetic backcrossed lines (SBL) is being utilized in wheat improvement (Dreisigacker et al., 2008; Li et al., 2018).

To fast-track development of breeders-friendly germplasm and eliminate several inherent bottlenecks in SHWs like F1 necrosis i.e. premature death of leaves, this parental stock is usually backcrossed to adapted wheat cultivars reforming SHWs to SBL (Ogonnaya et al., 2013; Singh et al., 2018; Babu et al., 2022). The first documented released wheat cultivar incorporating SBL of CIMMYT origin was Chuanmai 42 in China (Ogonnaya et al., 2013), though China has reportedly developed and released four cultivars named Chuanmai 38, Chuanmai 42, Chuanmai 43 and Chuanmai 47 so far (Yang et al., 2009). Several other countries like India, Pakistan, Ecuador, Argentina and Australia have also ventured in this direction and have received promising results. Despite advances in wheat breeding, the development of genotypes with stable performance under drought and heat stress remains a major challenge, largely due to the complex and interacting effects of these stresses on plant growth and yield. Moreover, the physiological variation and stress tolerance potential of synthetic backcross-derived lines (SBLs) have not been sufficiently characterized. Therefore, the present study was conducted to physiologically evaluate 200 SBLs under drought and heat stress conditions, identify superior and stress-tolerant lines, and select elite germplasm for incorporation into future wheat breeding programs.

Materials and Methods

Drought tolerance evaluation at germination stage

Seeds of 200 SBLs were disinfected with fungicide (Bevistin, 0.2%) and were sown in the Crop Physiology Research Lab, Crop Sciences Institute (CSI), National Agricultural Research Centre (NARC), Islamabad, Pakistan. The germinated seeds were then subjected to drought tolerance evaluation; 30 seeds of each line were placed in sterilized petri-plates covered at the bottom with a filter paper sheet. The filter paper sheet was moistened either with distilled water (control) or with equal amount of 20% Polyethylene glycol (PEG-6000; Sigma-Aldrich) solution as a stress. Seeds were germinated in dark

and were considered germinated when the plumule was at least 1 mm long. The germination percentage was determined after 7 days. Early seedling vigor was recorded after 15 days by measuring shoot length and root length.

Drought tolerance evaluation in greenhouse

In the greenhouse, the SBLs that were propagated under germination test were subjected to drought tolerance evaluation. Among 200 SBLs, 193 seeds of sprouted SBLs along with a check variety, NARC-2009, were sown in pots for drought tolerance assessment. The wheat plants were grown in pots (30 × 40 cm) size containing 10 kg sandy loam soil in a greenhouse under natural daylight and ambient day night temperature at NARC, Islamabad. The fertilizer was added to the soil before sowing as normal recommended dose of NPK (10 mg nitrogen kg⁻¹ as ammonium nitrate, 60 mg kg⁻¹ phosphorus as calcium hydrogen phosphate and 10 mg kg⁻¹ Zn as zinc sulphate). The pots were arranged in factorial, randomized, complete block design. Pots were regularly irrigated. Drought was applied at pre-anthesis till physiological maturity stage with one dose of 20% PEG-6000; Sigma-Aldrich. Five days after stress application, the flag leaf were excised and analyzed for physiological parameters.

Chlorophyll contents

Chlorophyll a and chlorophyll b contents were measured using Arnon (1949) method, by the following formula:

$$\text{Chl a} = [(12.7 \times 663) - (2.69 \times 645)] \times \frac{V}{W} \times \frac{1}{1000}$$

$$\text{Chl b} = [(22.91 \times 645) - (4.68 \times 663)] \times \frac{V}{W} \times \frac{1}{1000}$$

Membrane stability index (MSI)

Membrane stability index (MSI) was determined in flag leaves collected five days after the application of stress at the pre-anthesis stage. Flag leaves were excised from the wheat plants and immediately used for analysis. Leaf samples from the respective SBLs and the check variety NARC-2009 were subjected to electrolyte leakage measurement following the method of Sairam (1994). The electrical conductivity of the leaf tissue suspension was recorded before complete disruption of the cellular membranes (C₁). The samples were subsequently subjected to complete membrane disruption, and the final electrical conductivity was recorded as C₂. Membrane stability index was calculated using the following equation:

$$\text{MSI} = 1 - \frac{C_1}{C_2} \times 100$$

Where C₁ represents the initial electrical conductivity and C₂ represents the electrical conductivity after complete membrane disruption. MSI was expressed as a percentage (%).

Proline content

Proline content was measured using the method of Bates et al. (1973). Flag leaves were collected five days after stress application at the pre-anthesis stage from the respective synthetic backcross-derived lines (SBLs) and the check variety NARC-2009. Fresh leaf tissue was homogenized in 3% aqueous sulfosalicylic acid and filtered to obtain the extract. An aliquot of the extract was reacted with acid ninhydrin and glacial acetic acid and heated in a boiling water bath to develop the chromophore. After cooling, the reaction mixture was extracted with toluene, and the absorbance of the toluene phase was measured at 520 nm using a spectrophotometer. Proline concentration was determined using an L-proline standard curve and expressed as μmol g⁻¹ fresh weight (FW).

Relative Water Content (RWC)

Relative water content (RWC) was measured using the protocol of González and González-Vilar (2001). Flag leaves were collected five days after stress application at the pre-anthesis stage from the respective synthetic backcross-derived lines (SBLs) and the check variety NARC-2009. Fresh leaf samples were immediately weighed to determine fresh weight (FW). The samples were then fully hydrated in distilled water until maximum turgidity was achieved, gently blotted to remove excess surface water, and weighed to determine turgid weight (TW). Subsequently, the samples were oven-dried to a constant weight and weighed to determine dry weight (DW). RWC was calculated using the following equation:

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Where FW, TW and DW represent fresh, turgid, and dry weights respectively. The RWC was expressed as a percentage (%).

Heat tolerance evaluation

In the greenhouse, among 200 SBLs, the germinated seeds of 193 SBLs along with a check variety; NARC-2009 were sown in pots for heat tolerance assessment. The plants were grown in pots as described in drought tolerance assessment methodology. At post-anthesis (milky growth stage), the pots of three replications of each SBLs, each containing two plants were moved to a glasshouse for heat shocks for 5 h daily where temperature was maintained at 40 °C and 14/10 h day/night photoperiod, 50-70% relative humidity and illumination of 335 μmol m⁻² s⁻¹. Control and heat stress plants were regularly irrigated when required. For control plants, the temperature was 26 °C, illumination 300 μmol m⁻² s⁻¹ and humidity 62%

respectively. Three days after the heat shock treatments, flag leaf from the control and stressed plants was sampled for analysis of chlorophyll, proline and membrane stability index (MSI).

Statistical analysis

The experiment was performed in the layout of completely randomized design (CRD). The significant difference was calculated by employing analysis of variance (ANOVA) by factor factorial CRD design using two-way interactions. Further, the drought tolerance of the genotypes was calculated using the Heatmap; which was constructed on statistical software R (version 3.4.1) using physiological and yield traits of all studied SBLs. The relevant data was normalized by employing Z-score normalization prior to heatmap construction.

Results

Drought tolerance evaluation

The germination response showed significant variation ($p \leq 0.05$) among the tested genotypes (Fig. 1). Among the 200 SBLs, the emergence percentage ranged between 0% to 100% (Fig. 1). The highest germination percentage was recorded in 22 SBLs (1, 9, 15, 18, 26, 43, 49, 54, 56, 57, 75, 78, 100, 80, 81, 93, 130, 135, 142, 143, 155, 181 showed 80-100% followed by 26 SBLs (79-65%), while rest of the SBLs showed 0-40% germination. Similarly, the 47 SBLs having the highest germination showed greater seedling biomass. Further, the shoot length and root length were also recorded (Fig. 2). The highest root length (161 cm) was observed in SBLs 193. Whereas the highest shoot length (15 cm) was observed in SBL 24 and SBL 88.

The greenhouse study where plants were grown in pots revealed that on the basis of yield potential i.e. grain weight per spike, 1000 kernel weight and physiological trait (RWC), 31 SBLs were found to be drought tolerant (Fig. 3). These SBLs are: 6, 13, 17, 21, 24, 26, 32, 34, 35, 41, 42, 43, 44, 46, 47, 51, 53, 55, 57, 63, 64, 93, 98, 133, 146, 166, 181, 184, 186, 189 and 193. The relative yield reduction percentage of these lines ranged from 10 to 20% as compared to others which ranged from 25 to 50%. The SBLs specified above not only possessed high relative water contents but also showed higher yield attributes. Heatmap results of various morphological traits were correlated with each other as shown in the vertical top of the heatmap. A positive correlation of RWC with the thousand kernel weight further strengthened the role of RWC as a potent physiological screening criterion in studied SBLs under drought stress conditions (Fig. 3). Another important physiological parameter for screening of above mentioned SBLs in drought stress conditions was membrane stability index. The positive correlation observed between membrane stability index and thousand-kernel weight in the correlation matrix indicated that SBLs maintaining greater membrane integrity under drought

stress also tended to exhibit higher yield attributes. Conversely, SBLs with lower membrane stability were associated with reduced thousand-kernel weight. As far as chlorophyll a and b under drought stress is concerned, these two traits are negatively correlated. But MSI is positively correlated with the thousand kernel weight (Fig. 3).

Heat tolerance evaluation

In the greenhouse, plants were grown in pots and exposed to heat stress at post anthesis (milky stage). Based on yield and physiological parameters, 45 SBLs were found to have a minimum decrease in grain weight as compared to their control treatment (Fig. 3). These SBLs are 1, 9, 12, 22, 26, 44, 46, 47, 51, 52, 62, 72, 76, 77, 79, 89, 91, 92, 109, 112, 141, 144, 149, 151, 162, 165, 167, 168, 171, 174, 175, 176, 177, 178, 181, 182, 184, 185, 186, 187, 189, 190, 191, 192 and 193. Under heat stress, these lines showed the highest grain weight per spike and thousand kernel weights. Similar to drought, a heat map was constructed to visualize the association of SBLs with corresponding physiological and yield attributes in heat stress conditions. High proline accumulation in above mentioned SBLs under heat tolerance evaluation showed association with the yield attributes of respective lines in the heatmap. This was also confirmed by strong positive correlation between the proline levels and thousand kernel weights in the correlation matrix as shown in the vertical top of the heat map. On the other hand, there was no correlation observed between chlorophyll and membrane stability with respect to proline (Fig. 3).

Discussion

Agronomically improved synthetic wheat derivatives broaden the genetic diversity of bread wheat by introducing novel variation from primary synthetics and can outperform conventional cultivars under drought stress across diverse environments (Trethowan & Mujeeb-Kazi, 2008). Derived synthetics were estimated by quality and compared for their yield functioning as compared to their adapted recurrent parents in Mexico, under post anthesis drought stress given in the field by using limited irrigations. The results revealed that the derivatives were 23% higher in productivity as compared to their recurrent parents and 33% greater in yield than the check cultivar (Trethowan et al., 2003). Dreccer et al. (2007) determined a set of 156 synthetic backcrossed-derived bread wheat (SBWs) formulated by CIMMYT for rainfed environments and it was found that about 56% of the SBWs were superior in yield than that of the best local check cultivar. Reynolds et al. (2007) estimated two synthetic derived lines (SYN-DER) and their perennial parents (REC-PAR) under full irrigated and post-anthesis drought strain and reported that synthetic derivatives used 41% more water than that of their perennial parents, 24% higher in yield, produced 57% more biomass and had a 46% lower root and shoot ratio. Likewise in our studies, the highest germination percentage was recorded in 21 SBLs showed 80-100% followed by 26 SBLs (79-65%), while rest of the SBLs showed 0-40% germination.

As reported earlier, that heat stress directly affects the plant by hastening the normal pace of plant growth and development thus shortening the window of opportunity for intercepting sunlight and also reducing leaf area available for photosynthesis (Reynolds et al., 2012). Under intense stress, pollen development is affected that may result in increased number of aborted spikes and reduced grain number (Dolferus et al., 2011). Contrarily, drought stress leads directly to stomatal closure, thus reducing CO₂ fixation that ultimately leads to raise temperature of plant organs i.e. a localized heat stress. Higher intensity of drought leads to floret sterility, ultimately leading to shriveled grain growth (Barnabas et al., 2008). Most studies have interpreted these effects in terms of physiological processes, such as photosynthesis, transpiration, and stomatal conductance, or reduced plant growth at critical developmental stages, including emergence, stem elongation, heading, and grain filling. But literature lacks in encompassing the effects of abiotic stresses taking into consideration the yield components like number of spikelets, 1000 kernel weight etc. In wheat, yield is a function of available water (W), the efficiency of conversion of available water into assimilates (E) and the proportion of those assimilates into grains i.e. harvest index (H) under drought stress (Botwright Acuña et al., 2015; Sheng et al., 2022; Ghanem & Al-Farouk, 2024; Wang et al., 2025). Mohammadi et al. (2004); Khan et al. (2013, 2015) treated wheat plants a week after anthesis with 35/30 °C day/night for 3-5 days for evaluating heat tolerance in wheat. By following the same methodology, we found 45 SBLs to have a minimum decrease in grain weight as compared to their control treatment. Likewise in our study, proline besides being an amino acid also serves as an important osmoprotectant and scavenges hazardous reactive oxygen species generated under heat stress environments in wheat plants. SBLs with ability to accumulate higher proline levels are therefore able to withstand high heat stress levels. Relative water contents (RWC) were found to be the most predictive trait to screen wheat germplasm for drought tolerance.

Conclusion

Wheat is a major staple food crop in Pakistan, highlighting the need for targeted breeding efforts to enhance its nutritional quality, particularly iron and zinc concentrations, in a country facing a high burden of hunger and malnutrition. But it is one of the most climate (especially heat and drought stresses) prone crops. Modern wheat cultivars cannot cope with these issues, while wheat wild relatives, especially *A. tauschii* have wide variations for tolerance to these climatic factors and trace minerals in

wheat grains. Based on their overall agronomic and physiological performance, 30 synthetic derivatives were identified as drought-tolerant, while 45 exhibited heat tolerance. Relative water contents (RWC) were found to be the most predictive trait to screen wheat germplasm for drought tolerance and proline contents for heat tolerance evaluation.

Declarations

i. Ethics approval and consent to participate

Ethical approval and informed consent were not required for this study as it did not involve human participants, human data, or animals.

ii. Consent for publication

Consent for publication is not applicable.

iii. Data availability

All data generated or analyzed during this study are included in this article.

iv. Competing interests

Authors have declared that no competing interests exist.

v. Authors' contributions

J.U.D. designed the study and led the research group; M.S. performed the experiments and collected the data; Z.M. performed data analysis and interpretation; S.S. contributed to the experimental work and data collection; H.K. prepared the diagrams and figures. All authors contributed to the critical revision of the manuscript and approved the final version.

vi. Funding

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vii. Acknowledgement

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viii. SDGs addressed

Zero Hunger (SDG 2) and Climate Action (SDG 13)

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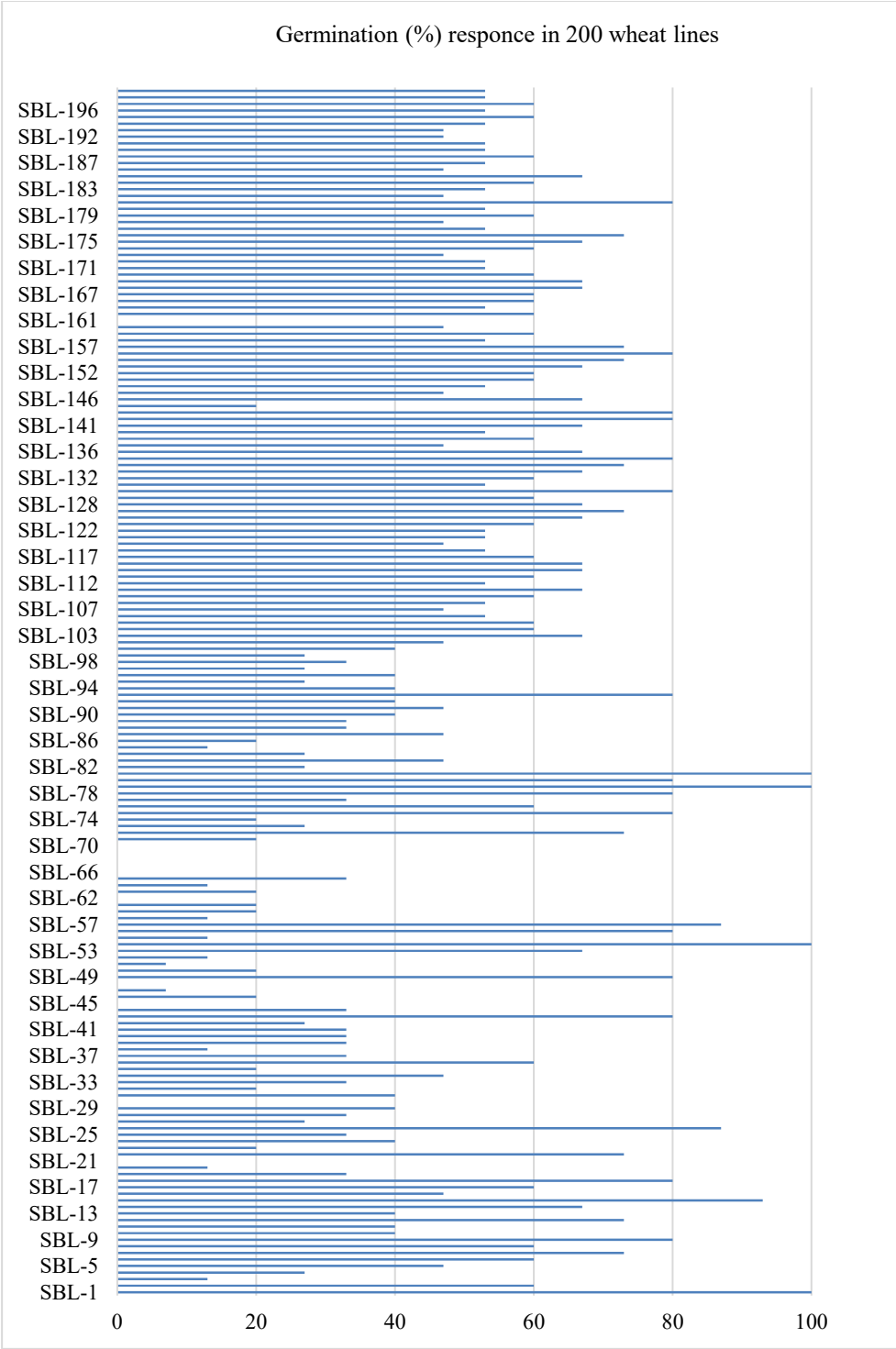


Fig. 1 Effect of 20% PEG-6000 on germination on wheat back-synthetic derivatives

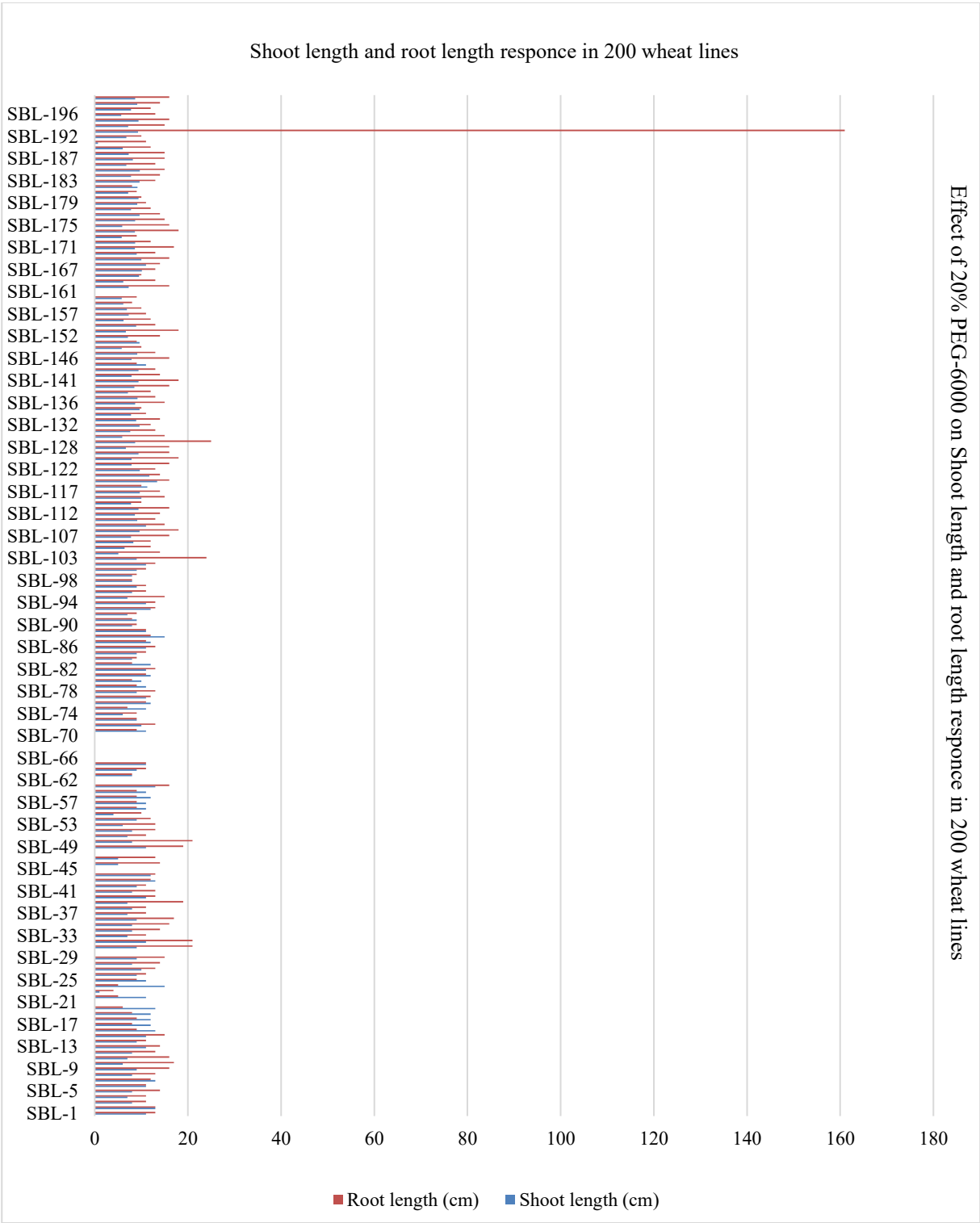


Fig. 2 Effect of 20% PEG-6000 on regeneration response on wheat back-synthetic derivatives

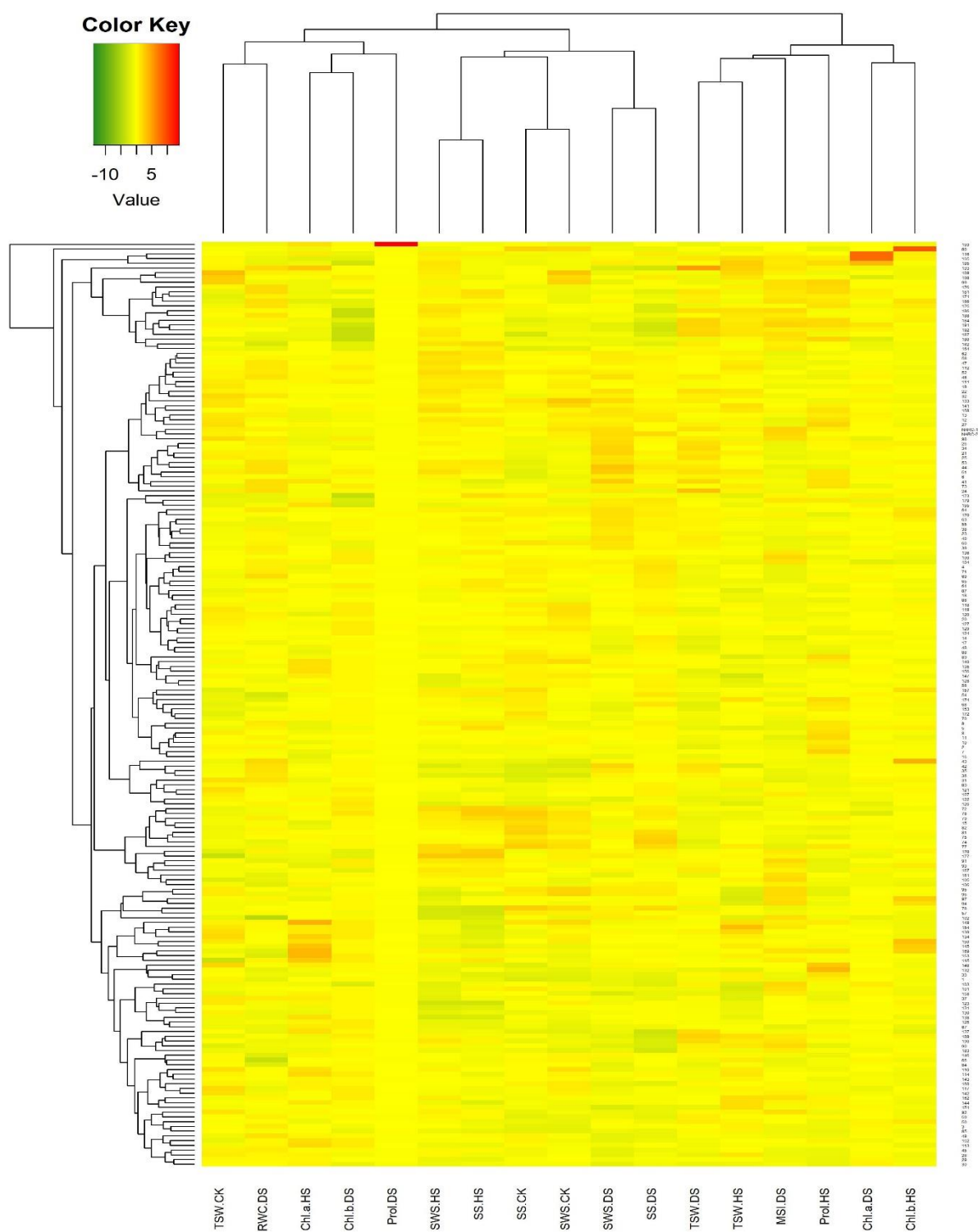


Fig. 3 Effect of terminal heat and drought stress on grain yield attributes and physiological traits of synthetic back-derived wheat lines under greenhouse conditions SS = seed weight/spike, SW = Seed weight, TSW =1000 seed weight, CK = Control, HS = Heat stress, DS = Drought stress.

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