

# Screening of Heat Stress Tolerant in Early Stage of Wheat Seedling using Morphological Parameters

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## ABSTRACT

Wheat (*Triticum aestivum*) is a Rabi crop and one of the dominant cereal, used worldwide as human staple food and livestock feed. Screening of heat stress tolerance in wheat requires various morphological parameters such as seed weight, seed size, imbibition rate, germination rate, seedling length, shoot length, root structure and its length, chlorophyll content, pH of soil, length of wheat crop. Here we studied six wheat varieties grown in various regions in India. Wheat is normally sown during November and harvested between March and April. For this, here we choose six varieties (DBW71, DBW88, DBW90, HD3059, S0072, and HD2851) of wheat to screen the heat tolerance among the varieties. During the analysis it was observed that DBW71, DBW88 and HD3059 are more heat tolerant than other. Various parameters such as chlorophyll content, shapes of leaves, size of shoots, roots morphology were explored for heat tolerant in wheat.

**Keywords:** Wheat (*Triticum aestivum*), Cereal crop, Heat tolerating and non-heat tolerating, Germplasm, Wheat, Seedling, Stress, Seed, Roots, Temperature.

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## INTRODUCTION

Wheat is a member of the Poaceae family and placed under genus *Triticum*. It is an annual, long day and self-pollinated plant. Wheat is the most important food cultivar at the global level than any other crop.<sup>[1]</sup> Temperature is one the most important climatic factors which effect the growth, development and yield of wheat.<sup>[2]</sup> Heat stress is a serious threat to crop production globally. As per the reports on climatic changes (IPCC 2014) temperature is globally rise<sup>[3-5]</sup> leading to approximately 0.3 and 1°C for years 2025 and 2100, respectively. Increasing temperature may alter the crop season however, crop gets mature earlier.<sup>[6,7]</sup> In 2016 Tripathi *et al.* also reported that heat stress has deleterious effect on crop production and its productivity which is very challenging for the world food security.<sup>[8]</sup> Although it is expected by increasing temperature,

prolonged wheat production will be very much affected. The Grain Filling duration (GFD) in wheat crop is also affected by temperature variation during the growing period related to grain development.<sup>[9-12]</sup> Since heat stress causes physiological changes by reducing its chlorophyll content that leads to leaf senescence in cool-season cereal species.<sup>[13,14]</sup> So, sufficient amount of nutrients and minerals are necessary for the plants growth under stress conditions.<sup>[15]</sup> At the stages of post-anthesis and pre-anthesis more than 31°C could decrease the rate of grain-filling and yield in wheat.<sup>[16-26]</sup> Abiotic heat stress can reduces photosynthetic capacity, imbalance between plant and water relations, hormonal imbalance, decreases of metabolic activities, production of oxidative reactive species, male sterility, reduction of pollen tube development and promotion of ethylene production in wheat.<sup>[13,27-31]</sup> Moreover in heat stress condition, the plants achieve fast growth, flowering, and maturation stage. Hence, the number of days to complete booting, heading, anthesis, and maturity in wheat were significantly decreased and varies among the genotypes. A healthy and green tillering stage was gradually reduces as the temperature increases. Tillering stage directly affect to number of spikes and

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spike linked to grain filling. However, heat stress reduces number of spike and grain size, which leads to yield loss of wheat.<sup>[32]</sup> Heat stress directly effects on ribulose-1, 5-bisphosphate carboxylase/oxygenase, Rubisco binding protein and Rubisco activase in wheat leaves.<sup>[33]</sup> It is also reported that *AP2/EREBP* superfamily plays crucial role in plant growth, development, biosynthesis of metabolites and biotic and abiotic stress responses.<sup>[34-36]</sup> In addition to this, it was confirmed that *AP2/ERF* genes were differentially expressed in wheat seedlings under the stress conditions such as heat, salt, and drought condition.<sup>[37,38]</sup>

Heat tolerance in wheat is also depends on the stage of seed germination.<sup>[39]</sup> During the initial 9 to 12 hr of imbibition, the imbibing wheat seed was found to exhibit substantial tolerance to high temperature relative to later times of imbibition. This initial high temperature tolerance gradually declines with increasing time of seed imbibition. High temperature decreased the photosynthesis rate, viable leaf area, shoot and grain mass, kernel weight and sugar content at maturity and reduced water use efficiency.<sup>[40]</sup> In the present study, we have selected the six important genotypes from north India region as shown in Table 1. We have investigated the morphological effect from 1D of sowing to 23 D of sowing on selected six varieties of wheat germplasm under heat stress conditions. Here we discussed morphological and agronomical aspects of D1 to D23 seed sowing to seeding stages such as shoot and root morphology, height, weight of fresh leaves and roots, wax coating in leaves, chlorophyll content and pH of soil of different varieties etc.

## MATERIALS AND METHODS

### Seed procured

We collected seeds of selected germplasm (DBW77, DBW90, DBW88, HD3059 and S0072) of wheat from reliable source in ICAR regional station Tepla, Haryana and other variety such as HD2851 was collected from a commission agent in New Grain Market of Shahbad, Kurukshetra, Haryana. All the varieties were stored at 4°C in our plant tissue culture laboratory, MMEC, MMDU (Mullana).

### Seed weight

Weight of 10 seeds of each variety was calculated to differentiate the wheat varieties (Table 1). Seed texture and physical condition of each variety were also recorded for further analysis.

**Table 1: Heat tolerance status of wheat varieties.**

S.no	Wheat varieties	Heat tolerance status
1	DBW 71	Suitable for very late sown conditions so better tolerant to heat stress.
2	DBW 90	This variety is developed for late sowing conditions so is better in tolerating heat stress.
3	DBW 88	It yields higher in late sown conditions that shows its better adaptation for tolerance to terminal heat stress.
4	HD 3059	Yields good in both late and very late sown conditions which reflects that it is better tolerance to terminal heat stress.
5	S 0072	Not able to tolerate heat.
6	HD 2851	Not heat stress tolerant as suitable for timely sown conditions.

### Preparation of soil

For seed sowing soil mixture was prepared in a ratio of 1:1:1 of sand, peat moss and vermicompost respectively. All the three components were mixed properly and filled into pots for further experimentation.

### Preparation of seeds

50 seeds of each variety were soaked in distilled water for 2 hr. Then 2 imbibed seeds were sowed in each lane with 20 biological replicates of each variety (Figure 1).

## RESULTS

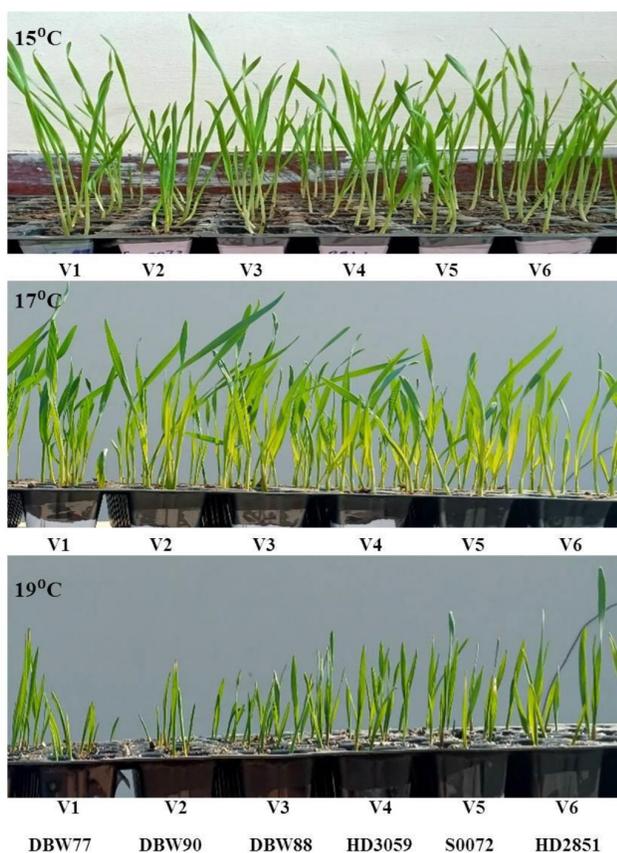
### Morphological characters of different varieties

#### Seed weight

Total 10 dry seeds of each wheat variety (V) were taken to measure the weight. After weight of all the varieties it was observed that there is variation in weight (Table 1). All the selected wheat varieties used for heat tolerant having nearly same seed mass but S-0072 is the one, which showed maximum seed weight (4.91gm/100 seeds) as shown in Table 1.

#### Seed sowing and analysis of V1 to V6 wheat variety

For screening purpose total six varieties were chosen from V1 to V6. Three different places were finalized for temperature variation for this experimentation (Figure 1). Tray1 is plotted in 15°C temperature in shadow area, Tray 2 in 17°C temperature in artificial light and Tray 3 in 19°C temperature in sunlight. Seeds were sown in plastic trays and each tray carrying 10 rows (R) with 10 columns (C) during month of February. Variety 1 (V1), Variety 2 (V2), Variety 3 (V3), Variety 4 (V4),



**Figure 1: Six wheat genotypes grown under three different temperature (15°C, 17°C, 19°C) in same soil mixture.**

Variety 5 (V5), Variety 6 (V6), were sown in C-1, C-2, C-3, C-4, C-5, C-6, respectively (Figure 1). Among the germplasm, DBW 88 (V2) was the one that grow fastest within 7<sup>th</sup> day of sowing at variable 15°C, 17°C, and 19°C temperature that showed seed germination of DBW 88 is susceptible under variable temperature. After 7 days of sowing, we saw seeds germination in V2 and about 10 days later on all the varieties have germinated (Figure 1).

### Root and shoot morphology

We measured length of roots and shoots of all wheat varieties with the help of measuring scale. At first, we measured shoot height of all the seedlings germinated in a row from the surface of soil. We named all six varieties as shown in Table 1. On 13 days of sowing an average height (in centimeters) of each shoot from each variety in a particular row growing at three different temperatures were observed. It was analysed that V3 and V4 were having maximum length among the six different germplasm as shown in Figure 1. As the temperature increasing from 15°C to 19°C, germinating time as well as seedling growth is gradually a decrease



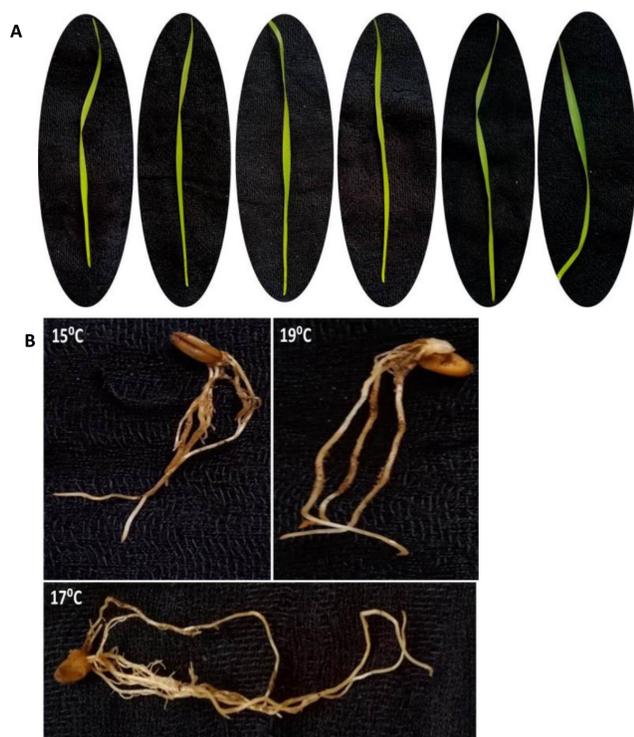
**Figure 2: Characterization of root and shoot of six different genotypes of wheat under seedling stage at different temperature.**

which shows many factors are involved at the time of germination and seedling which were affected above the optimum temperature. It is reported that several crops have three different factors to adapt under heat stressed conditions such as heat avoidance, heat tolerance and heat escape. During observation it was observed that V4 and V5 are the varieties which shown more wax coated on their leaf during seedling stage data not shown. Due to heat stress, seedling leaf margin were sharp may be for more transpiration (Figure 2).

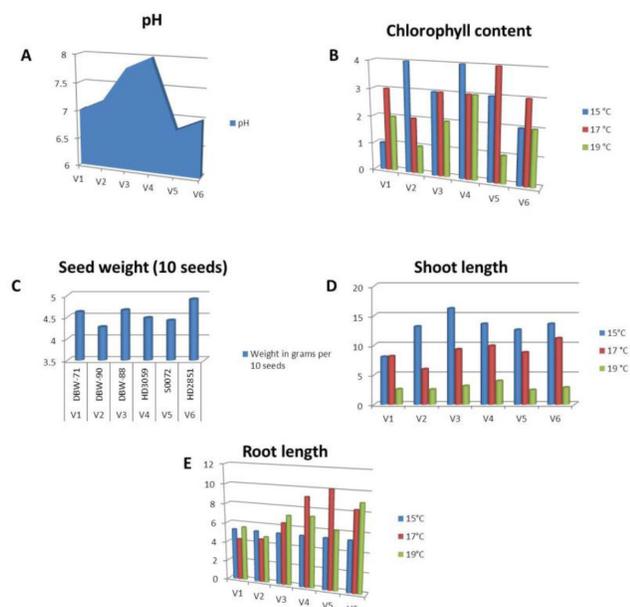
In our study leaf margin were sharpened in maximum varieties except V2 have round ends Figure 3A.

Root structure and length was also analysed after 24 D of sowing by uproot the whole seedling (Figure 2) and it was observed that the maximum variety grown at 17°C having more dense and maximum length than other temperature (Figure 3B).

In addition to this there are different soil agronomy, physiology and metabolic activities like pH of soil, chlorophyll content leaves, shoot length also vary under different temperature conditions (Figure 4).



**Figure 3A:** Leaf Margin in all the six variety. **3B** Representation of morphology of root and root length of wheat variety from different temperature.



**Figure 4:** Screening of various physiology and agronomical factor analysis. (A) pH of each variety from rhizosphere. (B) Chlorophyll content (C) Seed weight of 10 seeds from each variety (D, E) Shoot length and Root length.

## DISCUSSION

In wheat, seed mass is directly proportional to hardiness, seedling vigor, improved strand establishment and higher productivity.<sup>[44]</sup> Similarly plant growth depends

on seed size and its weight as in *Primula farinosa* heavy seeds produced larger rosettes, more flowers and seeds than lighter seeds produced plants.<sup>[42]</sup> To screen the heat tolerant potential of wheat we have choose 6 different varieties at three different temperatures. S-0072 is the one of the selected variety, which showed maximum seed mass (4.91gm/100 seeds). The large seeds of *P. racemosum* and *S. ciliata* have more capacity of seedling elongation than small ones.<sup>[43]</sup> DBW 88 is the important variety which is susceptible at different temperature (15°C, 17°C, 19°C). It is also reported that crossing of DBW 88\*C306 showing heat stress tolerance for yield and contributing in Bread Wheat.<sup>[44]</sup> Moreover DBW88 is a high yield variety of wheat and showed susceptible highest susceptible reaction against yellow rust.<sup>[45]</sup> It is also known that seed germination and seed dormancy was also affected by temperature in wheat.<sup>[46]</sup> As the temperature increasing from 15°C to 19°C, germinating time as well as seedling growth is gradually decreases which show many factors are involved at the time of germination and seedling which were affected above the optimum temperature. It was analysed that V3 and V4 were having maximum length among the six different varieties as shown in Figure 1.

It is reported that several crops have three different factors to adapt under heat stressed conditions such as heat avoidance, heat tolerance and heat escape. These factors are linked with different morphological and molecular trait (leaf rolling, waxiness, up regulation of stress responsive genes) which helps in tolerance and maintain the phenology of plants under heat stress conditions.<sup>[47]</sup> The waxiness of leaf surface reduces the loss of water and reflects the excess light in heat avoidance.<sup>[48]</sup> During observation it was observed that V4 and V5 are the varieties which shown more wax coated on their leaf during seedling stage data not shown. Due to heat stress, seedling leaf margin were sharp may be for more transpiration (Figure 2). The waxiness of leaf surface reduces the loss of water and reflects the excess light in heat avoidance.<sup>[48]</sup> During heat stress, plants reduced their leaf size and growth duration, leaf rolling, leaf shedding, thickening of leaves, high transpiration to maintained itself under heat stress damage conditions.<sup>[49,50]</sup> It is also observed that the maximum variety grown at 17°C having denser roots and maximum length than other temperature (Figure 3B). Similarly increased temperature could change the root structure and architecture in various plants.<sup>[51,52]</sup> There are different soil agronomy, physiology and metabolic activities like pH of soil, chlorophyll content, leaves, shoot length, shoot and root fresh weights, stem and

root lengths, leaf area, and shoot and root dry weights also vary under different temperature conditions.<sup>[53,54]</sup>

In plant growth different soil agronomy, physiology and metabolic activities like pH of soil, chlorophyll content leaves, shoot length also vary under different temperature conditions parameters, namely, shoot and root fresh weights, stem and root lengths, leaf length and leaf width, leaf area, and shoot and root dry weights were significantly decreased at 10 and 36°C compared with other treatments.

## CONCLUSION

Heat stress is a serious threat to crop production globally. Temperature is one the most important climatic factors which effect the growth, development and yield of wheat. We studied morphological parameter of shoot, root, their height, weight of fresh leaves and their roots, wax coating in leaves, and pH of seedling soil in different varieties at three different temperatures. We found that HD3059 and S0072 have more wax coated on their leaf during seedling stage. DBW88 and S0072 were having maximum length among the six different varieties. Moreover DBW88 is more susceptible at high temperature. Plant stress responses are very complex. The results achieved so far in this study indicate that many factors are affected from sowing to seedling stage in a definite hierarchy as the temperature increases.

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## CONFLICT OF INTEREST

The authors declare no Conflict of interests.

## ABBREVIATIONS

**1D:** one day of sowing **V1-** DBW77; **V2:** DBW90; **V3:** DBW88; **V4:** HD3059; **V4:** S0072; **V6:** HD2851; °C: degree celcius.

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