

# Assessment of Heat Stress Tolerance in Wheat (*Triticum aestivum*) Using Pollen Viability and Germination Test

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## ABSTRACT

Heat stress constitutes a key abiotic stress affecting Wheat productivity in light of climate change at present. The high temperatures prevailing during the critical phases of plant development such as anthesis and grain fill phase negatively impact pollen fertility and grain fill. Consequently, yield is reduced due to impaired fertilization. Hence, it is crucial to identify heat-tolerant wheat genotypes. The different screening procedures that can be used include pollen viability test and pollen germination test, which have been found to be relatively fast and accurate in assessing heat resistance at the reproductive stage. The pollen viability test is conducted using the differential staining procedure in determining the physiological condition of the pollen grain, while the pollen germination test determines the capacity of the pollen grain to form a pollen tube. This review will be centered on the effects of heat stress on the physiology of wheat plant focusing on its pollen biology. Specifically, the basic concepts and procedures involved as well as the importance of using pollen screens in crop breeding programs will be highlighted together with its strengths and weaknesses. In recent years, pollen-based screening techniques have received substantial attention owing to their simplicity, sensitivity, and efficiency. Viability tests of pollen grains, usually based on staining methods, including acetocarmine and TTC staining, give rapid insights into pollen quality and metabolism. On the other hand, pollen germination tests represent a functional approach that measures the potential of pollen grains to germinate and produce pollen tubes in controlled environments.

**Keywords:** Wheat, Heat stress, Pollen viability, Pollen germination, Heat tolerance, Screening, Abiotic stress, Reproductive stage, Climate change, Plant breeding.

## INTRODUCTION

Wheat (*Triticum aestivum*) happens to be one of the most extensively grown cereals across the world and forms a major source of food for many people around the globe. The nutritional value that wheat adds to our diet through carbohydrates, proteins, vitamins, and minerals makes it very important to us.

Wheat holds an important place in terms of food security and economy of nations such as India. Wheat production has become increasingly difficult owing to many abiotic stresses, heat stress being the most important one. In recent years, global warming has resulted in an increase in average temperatures coupled with an increased

frequency of extreme heat events. Such environmental changes have a very negative impact on agricultural production systems, especially for temperature-sensitive crops such as wheat. Heat stress refers to excessive temperatures above a critical temperature value for a sufficient period, resulting in the damage of physiological and developmental functions. During the reproductive stage, including the periods of booting, anthesis, and grain filling, the wheat becomes more vulnerable to higher temperatures due to irreversible negative effects on reproductive processes. Heat stress affects the physiological mechanisms, interfering with photosynthesis, increasing the rate of respiration, and leading to the instability of cell membranes. Moreover, it triggers protein denaturation and enzyme inactivation, preventing metabolic activity. On a biochemical level, heat stress promotes the formation of ROS that might damage cells. However, compared to other plant parts, pollen grains exhibit more sensitivity to heat stress. This phenomenon results from their sensitivity to any temperature alterations because of microsporogenesis and pollen function. In order to address the problems associated with heat stress, the discovery and selection of heat-resistant varieties of wheat are important. The traditional technique

performed under natural conditions is subject to fluctuations due to changes in the environment and takes a considerable amount of time. Hence, there is an increasing demand for fast, accurate, and reliable screening techniques. (R. T., & Hussein, M. H. 2021)

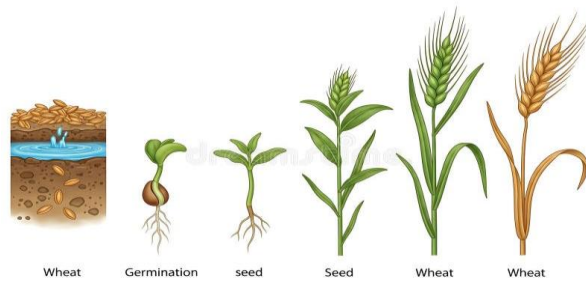


**Fig 1: Wheat (*Triticum aestivum*)**

The breeding of heat-stress resistant wheat genotypes is becoming a priority in order to preserve agricultural yields in the face of unpredictable weather trends. Traditional field-based evaluation procedures, despite their wide usage, are labor-intensive and affected by environmental variability. On the other hand, pollen-based testing protocols, including pollen fertility and pollen germination assays, are efficient, inexpensive, and convenient techniques that may prove useful in evaluating heat resistance from a reproductive point of view. Heat stress

effects on wheat are the subject of discussion in this review, with special consideration being given to pollen biology. The importance of testing pollen viability and germination as effective methods for assessing heat tolerance is also highlighted in this study, and their use in breeding wheat for heat tolerance is emphasized. Physiologically, heat stress impairs photosynthesis, speeds up respiration rate, and causes instability in cellular membrane structure. Additionally, the effect of heat stress is also reflected in the form of protein denaturation and enzyme deactivation, making it difficult for plants to conduct their biological processes properly. Biochemically, heat stress causes the production of reactive oxygen species, leading to oxidative stress on cellular

structures. Despite all the structural systems in plants being prone to heat stress, however, there are some more sensitive parts of a plant than others. Namely, pollen is especially sensitive to temperature changes, as it undergoes microsporogenesis and later functions. Heat stress during this stage leads to such consequences as low pollen viability, germination rate, and tube growth, resulting in poor fertilization, seed set, and grain yield. Consequently, since plant reproduction is one of the main factors influencing agricultural productivity, assessing its performance when exposed to heat stress serves as a good index of plant tolerance. (Masthigowda, M. H., Sharma, D., Khobra, R., Krishnappa, G., Khan, H., Singh, S. K., & Singh, G. P. 2022)



**Fig 2: Stage of wheat growth from seed to mature plant**

**Heat stress in Wheat**

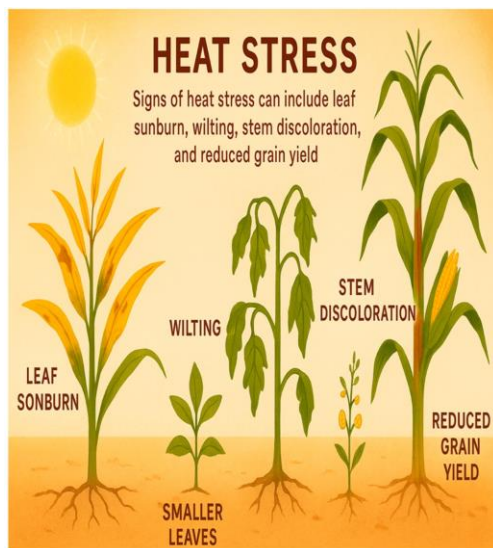
Among the many biotic stresses affecting the yield of wheat throughout the world, heat

stress is considered to be among the most crucial, especially keeping in mind the present-day condition of global warming. Heat stress occurs when the surrounding temperature goes beyond the optimal level needed for the proper growth and development of the plant. The optimal temperature for wheat ranges from 15°C to 25°C, whereas anything higher than 30-35°C causes considerable damage. Heat stress impacts wheat plants through morphological, physiological, biochemical, and reproductive aspects. Morphologically, heat stress causes

a reduction in plant height, premature aging, and poor grain formation. Physiologically, heat stress impairs photosynthesis by damaging chlorophyll content and influencing stomata conductance, but at the same time increases respiration rate, causing loss of energy. (Shenoda, J. E., Sanad, M. N. M. E., Rizkalla, A. A., El-Assal, S., Ali, R. T2021).



*Impact of heat stress on sensitive (left) and tolerant wheat varieties (right)*

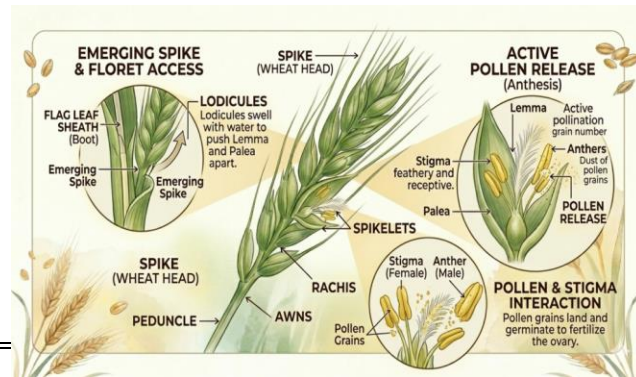


**Fig 3: Heat stress in wheat**

Heat stress affects cell structure at the cellular and biochemical level through protein denaturation, enzyme inactivation, and destabilization of cellular membranes. It triggers the overproduction of reactive oxygen species (ROS) that induce oxidative damage to lipids, proteins, and nucleic acids. The impact of heat stress on plants results in disruptions in normal cellular functioning and inhibiting their growth. It has been found that wheat's reproductive stage is vulnerable to the effects of heat stress. Heat stress adversely affects wheat booting, anthesis, and grain filling stages, thus reducing wheat yield significantly. One of the significant effects of heat stress is that it affects pollen formation and its viability and fertility. Heat stress interferes with microsporogenesis that leads to improper pollen formation and reduces their viability and germination rate. Ultimately, due to low fertilization and seed set, grain yield is affected. Apart from these, the grain-filling duration is shortened, thereby reducing grain size and weight. Overall, the effects of heat stress led to a decline in the production of wheat. Wheat plants employ various defense systems against heat stress. Wheat responds to heat by producing heat shock proteins, activation of antioxidant enzymes, and maintaining membrane stability. The effectiveness of the

wheat defense system. (Prasad, P. V. V., Boote, K. J., Allen, L. H., & Thomas, J. M. G. 2006)

**Table 1: Staining Methods for Pollen Viability**



**Fig 4: Flowering stage in wheat**

| Method                         | Principle        | Indicator Viability    | Advantages   | Limitations   |
|--------------------------------|------------------|------------------------|--|---|
| <b>Acetocarmine staining</b>   | Nuclear staining | Red-coloured pollen    | Simple and rapid                                     | Less accurate metabolically   |
| <b>TTC test</b>                | Enzyme activity  | Red formazan formation | High temperature stress indicates metabolic activity | Time sensitive enzymatic activity,  |
| <b>IKI staining</b>            | Starch detection | Dark blue/black pollen | Easy cellular structures and inexpensive             | Does not confirm germination  |
| <b>Pollen germination test</b> | Tube emergence   | Germinated pollen tube | decrease fertilization yields. Therefore, assess     | efficiency and grain. Requires controlled conditions assessing pollen viability |

**Pollen viability test**

The pollen viability test is an important physiological and cytological technique used to determine the proportion of living and functionally active pollen grains in a plant sample. In wheat, this test is extensively employed to evaluate the adverse effects of heat stress on reproductive development and to identify heat-tolerant genotypes. Pollen grains are highly sensitive to elevated temperatures, particularly during anthesis and fertilization stages, making pollen viability a reliable indicator of reproductive fitness under stress conditions. Ferris, R., Ellis, R. H., Wheeler, T. R., & Hadley, P. (1998).

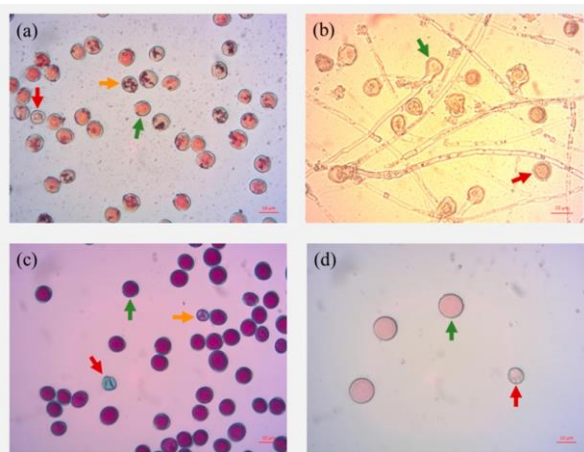
provides valuable information regarding the tolerance capacity of wheat cultivars under changing climatic conditions. The principle of pollen viability determination is based on differential staining techniques that distinguish viable pollen grains from non-viable ones. Viable pollens possess active enzymes, intact membranes, and metabolically functional cytoplasm that react positively with specific stains, resulting in characteristic coloration. In contrast, non-viable pollen grains fail to absorb stains properly because of loss of metabolic activity or cellular damage. Several staining methods are commonly used for pollen viability assessment in wheat and other crop plants, including **Acetocarmine staining**, **TTC**

(Triphenyl Tetrazolium Chloride) test, and Iodine–Potassium Iodide (IKI) staining. These methods are simple, rapid, and effective for screening large numbers of genotypes in breeding and stress physiology studies.

**Calculation:**

**Pollen viability percentage can be calculated using the formula:**

$$\text{Viability (\%)} = (\text{Number of viable pollen grains} / \text{Total number of pollen grains}) \times 100$$



**Fig 5: Pollen viability test Viable (dark red) and non-viable (unstained) pollen grains after acetocarmine staining.**

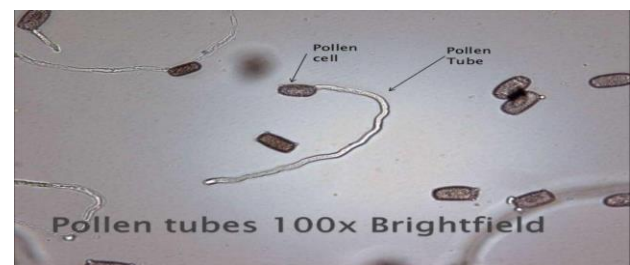
**Pollen Germination Test**

In Wheat, Pollen germination test is a significant technique which can be used in assessing the effectiveness of the pollen grain capability to germinate and form a pollen tube under favorable conditions. Pollen

germination is viewed as more reliable compared to other pollen viability tests as far as assessing fertilization ability is concerned, particularly in stress situations like high temperature. Pollen germination occurs where a pollen grain, after being placed on a suitable culture medium, takes up water and grows into a pollen tube. The pollen tube plays an instrumental role in transporting the male gametes to the ovule during fertilization. However, only the viable and metabolically active pollen grain has the capacity to germinate. This test usually involves the use of artificial media in the process of stimulating the growth of pollen. The main components of the media include sucrose as a source of energy, boric acid as a promoter of pollen tube growth, and calcium nitrate as an essential component in cell elongation. (Asseng, S., et al. 2015).

**Calculation :**

$$\text{Pollen germination \%} = (\text{Number of germinated pollen grains} / \text{Total number of pollen grain observed})$$



## Fig 6: Pollen Germination Tube

### Conclusion

High temperature is an important abiotic stress factor that has a strong influence on the physiology, development, and yield potential of Wheat plants. The negative effects of high temperatures on wheat physiology include poor pollen development, viability, and germination capacity, ultimately resulting in inadequate fertilization and low seed yields. Consequently, the study of the influence of heat stress on pollen biology becomes an important factor for improving the yield potential of wheat in changing climatic scenarios. Heat tolerance through pollen biology testing is an easy and rapid technique that has been found to effectively assess the heat tolerance potential of wheat cultivars. Techniques like pollen viability tests and pollen germination tests are highly reliable indicators of the physiological quality and fertilization ability of pollen grains. Where pollen viability test gives an estimation of the metabolic vigor of pollen grains, pollen germination test gives a better idea of their fertilizing ability. However, these techniques must not be utilized separately because they may fail to provide a comprehensive view of the performance of the plant in the field environment. In addition, by incorporating

the pollen technique with other physiological, biochemical, and molecular assays, selection could be made more accurate and efficient during breeding programs for wheat. To sum up, the utilization of techniques that evaluate pollen viability and germination is important when screening for heat tolerance in wheat varieties. With the use of this approach coupled with improved breeding technologies, the production of heat-resistant wheat can be achieved to provide sustainable crop production in the coming years amidst global climate change.( Asseng, S., et al. (2015).

### Future Perspectives

Recent advances in molecular breeding, high-throughput phenotyping, and omics technologies can be integrated with pollen-based screening methods to improve the identification of heat-tolerant wheat genotypes. Combining physiological screening with genomic selection and marker-assisted breeding may accelerate the development of climate-resilient wheat cultivars.

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