Comparative Evaluation of Pollen Viability and Germination under Heat Stress in Major Field Crops: Chickpea, Wheat, and Mustard

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ABSTRACT:

The rising temperatures are becoming an important threat for the crop yield. The developmental phase is especially sensitive to changes in temperature. Among several physiological factors that may be considered, pollen fertility and germination are reliable indicators of heat-stress, due to their role in fertilization. Aim of present research includes a comparative study of pollen viability and pollen germination concerning heat stress in three crop plants grown in the field including Chickpea, Wheat, and Mustard. The effect of heat stress in plant flowering stages is negatively related to the development of anthers, pollen sterility, and pollen germination, thereby leading to a reduction in seed production. From the comparative analysis that was carried out for the crops undergoing heat stress condition, it can be observed that the Chickpea crop is less resistant to heat stress than the Wheat and Mustard crops. On the whole, viability of pollen grains and pollen germination can be highly beneficial and efficient approaches in the selection of heat stress-resistant crops from diverse crops. The utilization of such reproductive characteristics in breeding programs may result in crops resistant to the effects of elevated temperatures. The negative effect of heat stress on reproduction in the case of the Wheat leads to a reduction in the viability and germination of pollen grains, which has a direct relation to the decrease in grain production in high temperatures. The Chickpea is also sensitive to heat stress resulting in decreased pollen fertility and germination of pollen grains caused by disturbances in carbohydrate metabolism in plants and, consequently, a lower content of sucrose in anthers. As for the Mustard, heat stress causes negative effects on pollen viability and fertilization, yet the sensitivity is genotypic.

Keywords: Heat stress, Pollen viability, Pollen germination, Chickpea, Wheat, Mustard, Reproductive stage, Pollen fertility, Seeds formation, Yield, Susceptibility by genotypes, Screening methods, Crop development, Temperature stress.

INTRODUCTION

The increasing effect of global warming is one of the key problems being faced by agriculture because of its effects on crops productivity. Heat stress, which refers to an environmental state where the temperature surpasses the optimal levels for the plant's development, can have grave consequences in terms of physiology and biochemistry of the plant, particularly during the reproductive phase of development (Wahid *et al*., 2007; Bita and Gerats, 2013). Among other development phases, the reproductive stage is sensitive to heat stress.

1.Heat Stress and Crop Productivity

1.1 Concept of Heat Stress and its Definition

Heat stress is experienced by plants under high temperatures that are not within their normal range, which affects their metabolism processes and internal balance (Wahid *et al.*, 2007) This condition may either be transient or lasting, depending on the external environment.

Fig. 1 – Normal plant compared to heat stressed plant

1.2 Effects of Heat Stress on Plant Development and Yield

Heat results to negative impacts on photosynthesis, assimilation, and respiration rates. Such effects reduce biomass production and yield (Farooq *et al.*, 2011). Heat stress affects yield most significantly when it happens during reproduction.

2. Sensitivity of Reproductive Stage to Heat Stress

2.1 Flowering and Fertilization Events

Flowering and fertilization events include many processes that are sensitive to any variations in temperatures (Hedhly, 2011). The heat will make the synchrony between the dehiscence and stigma receptivity difficult.

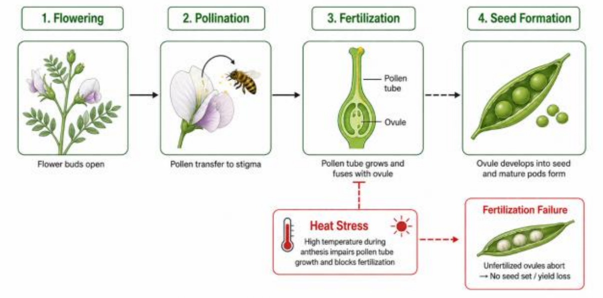


Fig. 2- Heat disrupting the sequence of Flowering → Pollination → Fertilization.

2.2 Anther and Pollen Formation

Heat stress inhibits anther dehiscence and pollen formation and release, reducing the chances for successful fertilization events. Deformed pollen grains are common under heat stress situations.

3. Pollen Viability and Germination as Screening Traits

3.1 Pollen Viability

Pollen viability refers to the capacity of the pollen grains to survive and retain functionality, which is required for effective fertilization (Hedhly *et al.*, 2009). This trait can be determined using methods such as acetocarmine and TTC stains.

3.2 Pollen Germination

Pollen germination can be defined by the capability of the pollen grains to develop a pollen tube, allowing fertilization to occur. This means that the germination of pollen can be considered a feature indicative of successful reproduction (Shivanna and Rangaswamy, 1992). Elevated temperatures have been shown to impact pollen tube growth, hence germination rates.

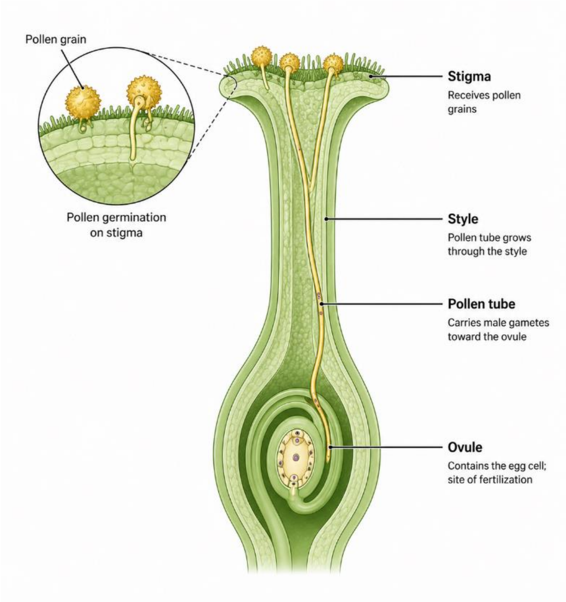


Fig. 3 – Pollen Germination and Pollen Tube Development

3.3 Significance in Screening Heat Resistance

Pollen viability and germination are significant screening traits that determine heat-tolerant varieties because they represent reproductive fitness.

4. Heat Tolerance of Plants

4.1 Chickpea

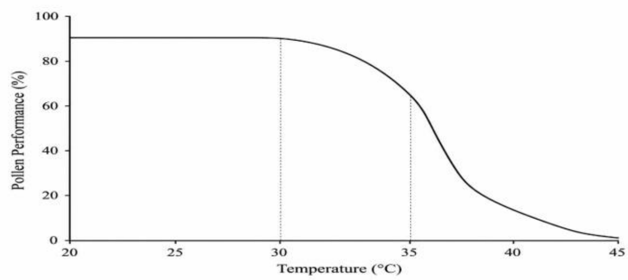
Plant Chickpea is highly sensitive to heat stress especially at flowering stage, leading to poor pollen viability, poor germination and pod formation (Nayyar et al., 2005; Kaushal et al., 2013).

4.2 Wheat

Plant Wheat is moderately sensitive to heat stress, causing poor pollen fertility and poor seed setting (Prasad et al., 2008; Farooq et al., 2011).

4.3 Mustard

Mustard plant has moderate heat tolerance, although heat stress may influence pollen viability, fertilization



Graph 1 - Threshold effect of heat.

5. The Need for Comparative Evaluation and Screening

5.1 Importance for Breeding Programs

Using the pollen traits for screening helps in identifying heat tolerance at an early stage, thereby promoting plant breeding programs (Zinn *et al.*, 2010).

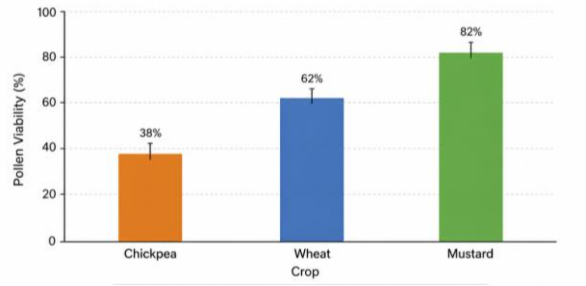
6. Rationale of the Study

6.1 Significance of Pollen Screening

Yield screening is a tedious process and is highly dependent on environmental factors; pollen screening offers a quick and efficient method.

6.2 Coverage of Comparative Analysis

Comparative analysis of Chickpea (*Cicer arietinum*), Wheat, and Mustard contributes to the knowledge of specific reactions in each variety and the selection of adaptive crops.



Graph 2 – Pollen Viability under Heat Stress in different Crops

MATERIAL REQUIRED AND METHOD

1.Experimental Site and Plant Material

This experiment was carried out under controlled laboratory and in Rama University, Agriculture Field during rabi season. Three different crops, which include Chickpea (*Cicer arietinum*), Wheat, and Mustard were selected for the current experiment. Seeds of these different crops were collected from a seed certification agency and planted according to the suggested agricultural method.

2. Experimentation & Application of Heat Stress Condition

2.1 Design of the Experiment

The experiments were conducted using CRD design with each plant crop having three replications. Plants were allowed to grow under optimum growing conditions until flowering stage.

2.2 Introduction of Heat Stress Condition

Application of heat stress condition was carried out at the flowering stage, the most critical stage in the reproduction process of the plants. Heat stress condition was created through increased temperature (35-40°C) for specific periods (4-6 hours a day) either through growth chambers or manipulation of sowing times when the plants experience high temperatures. The control plants were grown at optimum temperature (25-30°C).

3. Collection of Pollen Samples

Mature flowers from each of the crops were obtained during the morning time period. The anthers from the flowers were excised and processed directly for pollen examination. This helped to ensure the viability of the pollen.

4. Evaluation of Pollen Viability

4.1 Staining Test

The viability of pollen grains was evaluated through the use of the staining tests like acetocarmine staining (Shivanna and Rangaswamy, 1992).

4.2 Process

Some of the pollen grains were put on a slide and stained with 1% acetocarmine.

The slides were then observed through a light microscope where the viable pollen showed a darker stain while the non-viable ones did not show any colour at all.

4.3 Formulation

The percent viability of the pollen grains was calculated using the equation below:

Pollen Viability (%) =

Total number of pollen grains /
Number of viable pollen grains ×100

5. Estimation of Pollen Germination

5.1 Preparation of Germination Medium

The germination test of pollen was done using the medium formulated by Brewbaker and Kwack consisting of sucrose, boric acid, calcium nitrate, magnesium sulphate, and potassium nitrate (Brewbaker and Kwack, 1963).

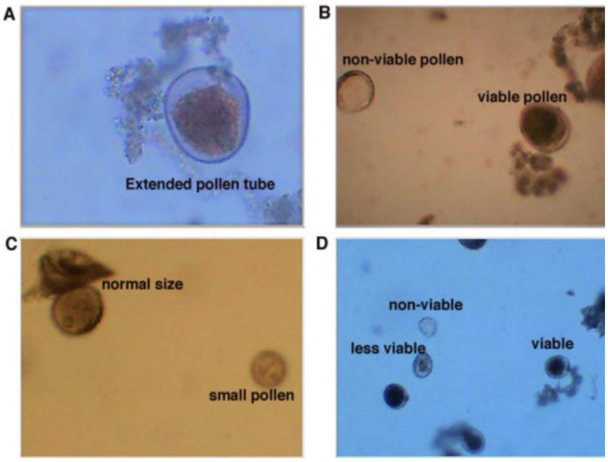


Fig. 4

A - Extended Pollen Tube.

B - Viable and Non-Viable Pollen.

C - Size of the Pollen.

D – Less Viable Pollen.

5.2 Procedure

A drop of the germination medium was put on a clean glass slide and the pollen grains were sprinkled on it. The slides were stored in a humidity-controlled box maintained at an ideal temperature range (25-30°C) for 2-4 hours. Lastly, the slides were observed through a microscope.

5.3 Germination Criteria and Calculation

The criterion for pollen germination was set that any pollen grain was germinated if the length of the pollen tube exceeded its diameter. The calculation was as follows:

Pollen Germination (%) =

Total number of pollen grains / Number of germinated pollen grains x100

DISCUSIONS RESULTATS

6. Data Collection and Statistics

The data concerning pollen viability and germination was collected from different microscopic views per replication. test was performed to establish the significance of differences between treatment and crop types (Gomez, 1984). The standard deviation and critical difference (CD) values were calculated using a 5% level of significance.

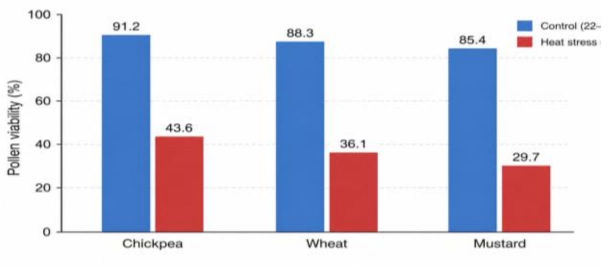
7. Comparative Analysis

Comparative study was carried out on Chickpea (Cicer arietinum), Wheat, and Mustard with respect to heat stress in the form of percentage viability of pollen and percentage germination of the pollen.

7.1 Effect of Heat Stress on Pollen Viability

Heat stress effect was found to have a major influence on the viability of pollen of the three plants. In case where heat stress effect does not exist under favourable conditions, the viability of Cicer arietinum, Triticum aestivum, and Brassica napus is relatively high; however, owing to heat stress taking place at temperatures between 35-40°C, there is a large drop in viability (Saini and Aspinall, 1982). Concerning the viability of pollen, it was found out that the viability of Cicer arietinum is higher than that of

Triticum aestivum, but lower for Brassica napus.



Graph 3 – Effect of Heat Stress on Pollen Viability on selected Crops under normal and Stress Condition

7.2 Effect of Heat Stress on Pollen Germination

Heat stress significantly impacted pollen

Crop	Germination (%) Control	Germination (%) Heat Stress	Reduction (%)
Chick pea	80	40	50
Wheat	82	55	32.9
Mustard	85	65	23.5

germination percentage for all crops. The pollen germination percentage was very high in case of an optimal temperature condition. However, due to heat stress, there was a considerable drop in pollen germination percentage (Hedhly *et al.*, 2009). again Chickpea (*Cicer arietinum*) demonstrated maximum reduction in terms of pollen germination percentage, followed by Wheat. In contrast, Mustard retained its pollen germination percentage in spite of the heat stress. heat stress also, pollen tube elongation was restricted (Prasad et al., 2017).

Table 2 - Effect of Heat Stress on Pollen Germination

7.3 Response of selected crops for Heat Stress

From the above study, it was found through comparative study that there are different responses of the three plants to heat stress. Chickpea (Cicer arietinum) plant shows high sensitivity to heat stress because of low pollen viability and germination. In case of wheat plants, there was moderate sensitivity while for mustard plants there was high tolerance. Above results prove that the sensitivity varies from species to species towards heat stress (Bita and Gerats, 2013).

7.4 Relationship between Pollen Viability and Germination

From the results obtained, it can be stated that pollen viability is positively correlated to pollen germination across all experiments conducted. Plants with high pollen viability levels had corresponding high levels of pollen germination and, thus, pollen viability and pollen germination levels are closely related and can serve as parameters for determination of reproductive fitness under stress (Zinn et al., 2010).

7.5 Statistics

A one-way analysis of variance showed there was statistical significance at the 5% probability level on the influence of heat stress on pollen viability and germination levels. Statistical significance was evident across the different plants tested as well as control and stressed crops; showing temperature was a key factor in reproduction.

CONCLUSION

Results from this research confirm what has been shown previously: Heat stress has a large negative effect on reproduction in crops grown in fields by producing large decreases in viability and germination of pollen. High temperatures during the flowering stage resulted in substantial decreases in pollen performance, indicating that reproductive processes are one of the stages in the life cycle of plants which is most sensitive to heat. Pollen germination was significantly more sensitive than pollen viability among the two parameters measured, suggesting that pollen germination is the most reliable indicator of heat stress damage. Pollen viability and germination are reduced due to heat stress through a variety of physiological and biochemical processes. When temperatures rise, protein denaturation, decreased enzymatic activity, and increased reactive oxygen species production occur which together disrupt cellular metabolism. When these disruptions occur, pollen tube growth is also disrupted due to changes in the energy supply, membrane stability, and cell wall formation, reducing both fertilization success rates and seed set. The comparative evaluation of the selected field crops/genotypes showed a significant amount of variability in how they each responded to heat stress. Therefore, some crops/genotypes maintained relatively higher pollen viability/germination under stress conditions suggesting the presence of inherent mechanisms that confer heat tolerance. The experiment has revealed the significance of pollen characteristics, particularly pollen tubes, which can act as effective indicators of resistance against harsh temperatures of the environment. In simpler terms, these physiological features will aid breeders to come up with temperature tolerant varieties in much lesser time. Since there is a growing tendency for climate change causing uncertainty in temperature variations, this innovation will become very important.

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