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Wheat Pollen Viability and Germination at Different Storage Temperatures

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Abstract

In technical breeding research and genetic resource conservation, the storage of vegetative plant components, including pollen, has become increasingly significant. Wheat pollen cannot sustain their viability for an extended period of time, in contrast to many other plant species. In this study, pollen viability and germination rate assed at three storage temperatures (room temperature, +4 ^{o}C and -18 ^{o}C) and different 0 (fresh), 48, and 96 h of storage duration. Seven wheat varieties of species and growth habits were used. Pollen viability was determined using the TTC staining test, and pollen germination was determined by "agar in plate" method. In the fresh test, germination was 43% and pollen viability was 77%. Pollen viability dropped to 44% at 4 ^{o}C, 12% at -18 ^{o}C, and 0% at room temperature after 48 hours of storage. After being stored at 4 ^{o}C for 48 hours, germination dropped to 22%. At -18 ^{o}C and room temperature, no germination was seen. Wheat pollen entirely loses its viability and germination potential after 4 days, despite the fact that storage at 4 ^{o}C produces better outcomes than storage at ambient temperature and -18 ^{o}C.

Key words: Wheat pollen, Wheat pollen storage, Viability, Germination

Introduction

The short lifetime of wheat pollen is defined by its quick germination following anthesis. Temperature, relative humidity, light, and wind are some of the environmental variables that affect wheat pollen preservation. The goal is to effectively explain the procedure

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keeping wheat pollen in storage, especially for breeding (Khan et al., 2024). Following the second pollen mitosis, the male gametophyte, also known as the pollen grain, is liberated from blooming plants. Mature wheat pollen grains are tricellular, have a high moisture content, and have a brief lifetime. Tricellular pollen's respirator activity, which requires effective pollination, is the cause of its restricted viability (McCormick, 2004).

The strength and viability of pollen grains are essential for agricultural plant pollination, and the collecting stage is a critical factor in this process. Pollen from closed flowers is thought to be the best for preserving high viability because of its immaturity and less vulnerability to contamination (He et al., 2017). Pollen viability is important for hybrid seed production (Longin and Reif, 2014). Stainability, germinability, and fertilization ability are used to assess pollen viability. *In vitro* germination is a frequent viability assessment in genetic improvement initiatives, but each species requires a different set of methods and culture medium. Pollen viability is significantly impacted by storage conditions, especially temperature. Cell membrane damage from ice crystal formation can be avoided by ultra-low temperature (cryo) preservation, which calls for significant dehydration. Finding the ideal preservation temperature is crucial since it might be difficult to get the proper moisture content and thawing technique (Jumrani et al., 2018; Jia et al., 2022).

For breeding and genetic studies, pollen storage is essential. There is little information about storing wheat pollen at low temperatures; one research found that it could be preserved for a day at 5 °C. The lifespan of pollen varies greatly among plant species, genotypes, and cultivars. For example, in field circumstances, the pollen of Agrostis stolonifera L. rapidly loses vitality, while the pollen of maize becomes non-viable in two hours (Mukti, 2014; Banisab et al., 2017; Masthigowsa, 2022). Understanding how long pollen lasts after being stored in various settings is essential for breeding programs, genetic conservation, artificial pollination, and self-incompatibility. the storage of pollen as a genetic resource is a useful method (economically, storage of excess material in small areas, etc.).

Wheat pollen rapidly loses its vitality immediately after dispersal (Impe et al., 2020). Long-term pollen storage will remove the necessity for synchronized flowering in

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parents, the need to grow father plants during the same crossover time, the availability of parent plants produced in diverse settings, and reliance on weather in wheat breeding projects. Labor, expenses, and breeding danger are eliminated as a result. A crucial procedure for guaranteeing pollination, sustaining regular date palm bearing, particularly for early season flowering female cultivars, and conserving biodiversity is the storage of pollens while keeping increased viability and germinability (Choudhry et al., 2014). It is still unknown why wheat pollen has such a limited lifespan. The primary determinants of pollen viability are temperature, humidity, and plant type (De Storme and Geelen, 2014). Pollen's storability can be better understood by looking at its structure and the physical and chemical changes it experiences after dispersal (Impe, 2022). One of the most crucial areas of research is figuring out the storage temperature. The aim of this study is viability and germination tests to determine how wheat pollen reacted to different storage temperatures.

Materials and Methods

Plant material

Pollen was used in the experiment obtained from winter-sown wheat under field conditions during the 2019-2020 period. The plots were arranged as 2 m 3 rows. 'Super Ekin'™ (13.25.5 + (10SO3) + ME) base fertilizer was applied at 20 kg da^{-1} and 25 kg da^{-1} Ammonium Nitrate (AN)) top fertilizer was applied in spring. The plots were irrigated depending on rainfall. Bezostaja-1 (*T. Aestivum* L.), Ç-1252 (*T. Durum* Desf.), Kocabugday (*T. Durum* Desf.), Tosunbey (*T. Aestivum* L.), Esperia (*T. Aestivum* L.), Altın 40/98 (*T. Durum* Desf.), Emmer (*T. Dicoccon*) wheat varieties were used in the experiment.

Method

Pollen samples were taken from the middle row, when flowering was 40-80%, by cutting the stems 20 cm below the spike. The spikes were immediately brought to the laboratory

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and placed in glasses filled with water (Figure 1). Pollen was poured by shaking (Figure 2). To determine the quality of wheat pollen, viability and germination tests were performed in four replications. The first test was performed immediately with wheat pollen (Fresh). The other parts were divided into 3 Petri dishes and stored at room temperature (22 °C ±2), in a refrigerator (+4 °C ±2), in a deep freezer (-18 °C ±2) without humidity control. The second and third viability and germination tests were performed after 2 days (48 h) and 4 days (96 h), respectively.

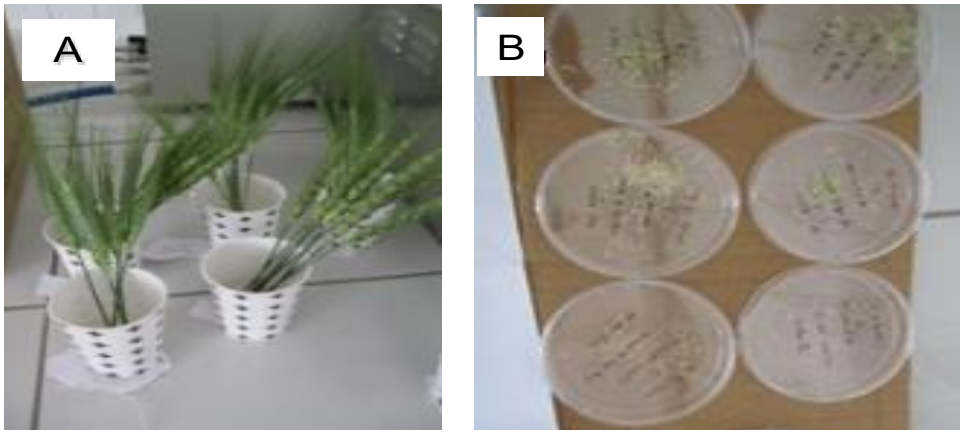

Figure 1. Flowering wheat spikes (A) and wheat pollen in Petri dishes (B)

1% 2,3,5 triphenyl tetrazolium chloride (TTC) staining was used to assess the pollen's viability level. Ten milliliters of TTC solution were made by dissolving 0.1 grams of TTC in one milliliter of pure water and 6 grams of sucrose in nine milliliters of pure water. The two solutions were then combined (Resende et al., 2014). Following the addition of one drop of TTC to each cultivar's slide, pollen was scattered over the solution using a water colour brush. A coverslip was then placed over the slide, and each area was split into four sections before being kept in an incubator set at 21 ^{o}C. Pollen viability was assessed using a light microscope two hours after the pollen was sown. At a magnification of 40, pollen that was stained red was considered "viable," while those that were not stained were classified as "non-viable."(Figure 3). Pollen germination experiments were conducted using the "Agar in plate" method (Guclu and Koyuncu, 2017; Guclu et al., 2018). The medium comprising 1% agar + 15% sucrose + 5 ppm boric acid was identified as the germination media due to the early studies. Each petri was divided into 4 regions, and 100 pollen samples were selected from each region. Pollen tubes long at least as its diameter was considered to be “germinated” (Guclu et al., 2020). Germinated pollen is

shown in Figure 4 (B). Variance analyses of the results were performed using the SPSS statistical program, and the difference between the groups was determined according to Duncan's test.

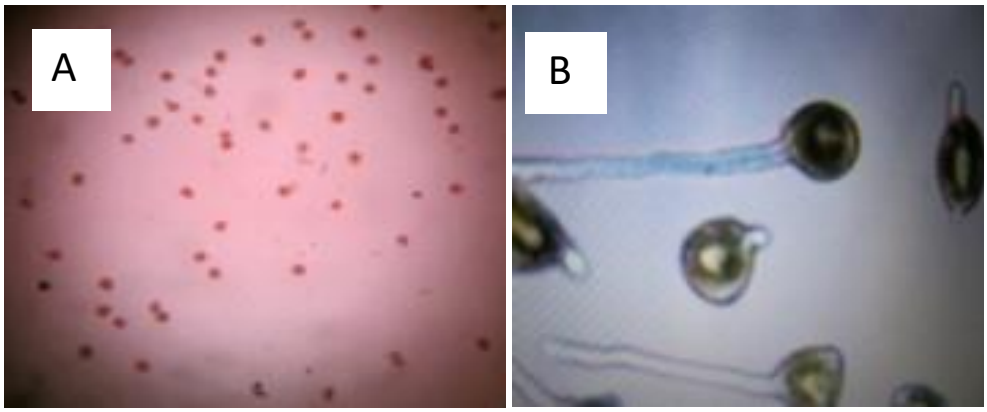

Figure 2. Representative image of pollen viability (A) and germination (B)

Results

Because of the analysis of the data obtained, the differences between varieties and storage conditions were found to be statistically significant (P<0.05) (Table 1). The results of pollen viability and germination tests at fresh and 48 h (+4 and -18 ºC storage) are shown in Figures 5 and 6. After 48 h of storage at room temperature, pollen viability and germination could not be determined. For 96 h (room temperature, +4, and -18 ºC), viability and germination were not determined.

Table 1: Results of variance analysis of pollen viability and germination test in wheat

	Viability (%)				Germination (%)			
Source	df	Mean Square	F	Sig.	df	Mean Square	F	Sig.
Variety	6	1375.107	12.688	.000	6	611.946	4.260	0.002
Storage con.	2	59677.426	550.623	.000	1	6237.161	43.423	0.000
Var. X Storage con.	12	377.793	3.486	.000	6	33.327	0.232	0.964
Error	147	108.382			42	143.637		

In the fresh viability test, pollen viability among the varieties varied between 62% and 83.5% (Figure 5A). The highest viability was determined in the Bezostaja-1 and Esperia varieties (83.5%), whereas the lowest viability was determined in the Kocabugday varieties (62%). The average viability was 77% (Figure 5B). In pollen stored at +4 ºC for

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48 h, the viability of the varieties decreased significantly compared with the fresh test. The lowest was found in the Bezostaja-1 variety (30%) and the highest in the Emmer variety (47.5%). The lowest value was observed in the Kocabugday variety (1%) and the highest value was observed in the Esperia variety (26.5%) in pollen stored at -18 ^{o}C for 48 h. The decrease in viability at -18 ^{o}C was higher than at +4 ^{o}C. While this decrease was around 43% in pollen stored at +4 ^{o}C, it was around 85% in pollen stored at -18 ^{o}C.

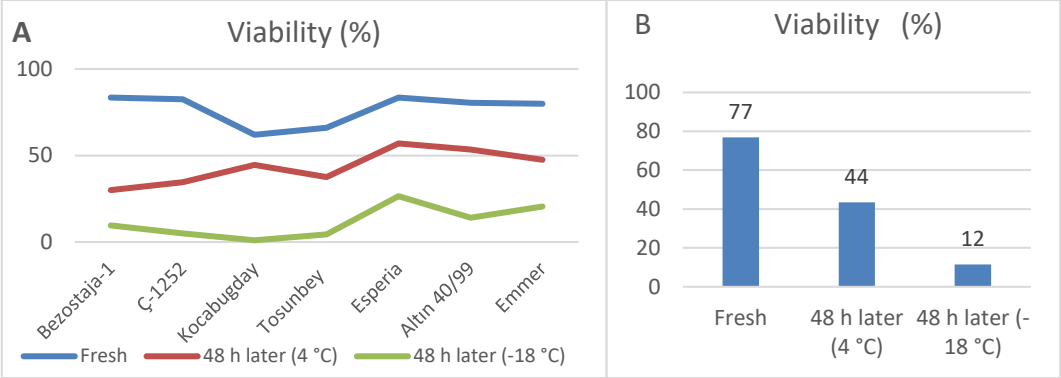

Figure 3. Viability of wheat pollen according to varieties (A) and storage duration (B)

In the fresh germination tests, the average pollen germination of the varieties varied between 31% (Ç-1252) and 58% (Esperia) (Figure 4A). The average germination percentage was found to be 43% (Figure 4B). In pollen stored at +4 ^{o}C for 48 h, germination of all varieties decreased, the lowest value was realized in the Kocabugday variety (15%) and the highest value was realized in the Emmer variety (28%). The average germination rate was 22%. Between the fresh and second tests (48 h at +4 ^{0}C), germination losses in all varieties were (15-27%) and the average was around 50%. No germination was observed in pollen stored at room temperature and -18 ^{0}C for 48 h.

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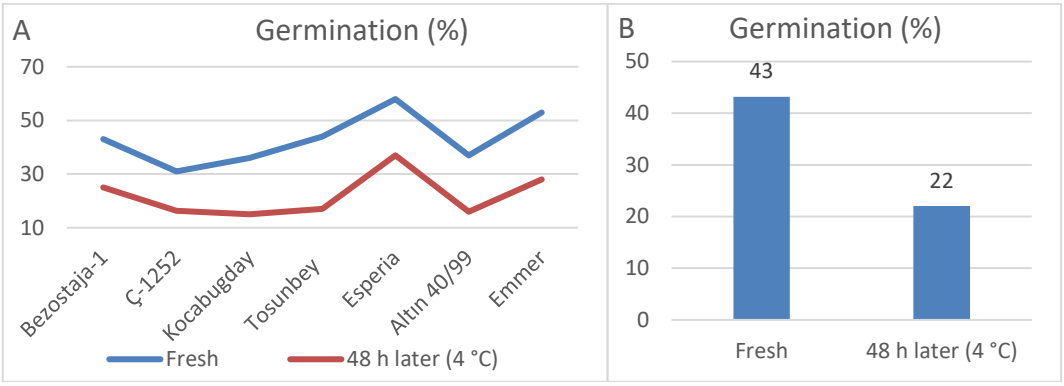

Figure 4. Germination of wheat pollen according to varieties (A) and storage duration (B)

The difference between the viability and germination ability of wheat pollen was evident in the first and second tests (Figure 5). On average, 77% of the pollen showed viability and 43% showed germination ability. The difference between viability and germination ranged between 22% and 52% in the first test, with an average of 34%. In other words, only 2/3 of the live pollen has germination properties. In pollen stored at 4 ºC for 48 h, viability decreased to 44% and germination decreased to 22%. The difference varied in a wide range between 5% and 38%. In general, it is possible to say that there is a parallel between viability and germination. In other words, germination was also high in varieties with high vigor. However, this difference was very high in Altın 40/99 and Kocabugday cultivars, whereas it was the least in the Bejostaja-1 cultivar. The decrease in germination compared with vigor was 44% in the first test and 48% in the second test. As time passed, the difference increased by 4%.

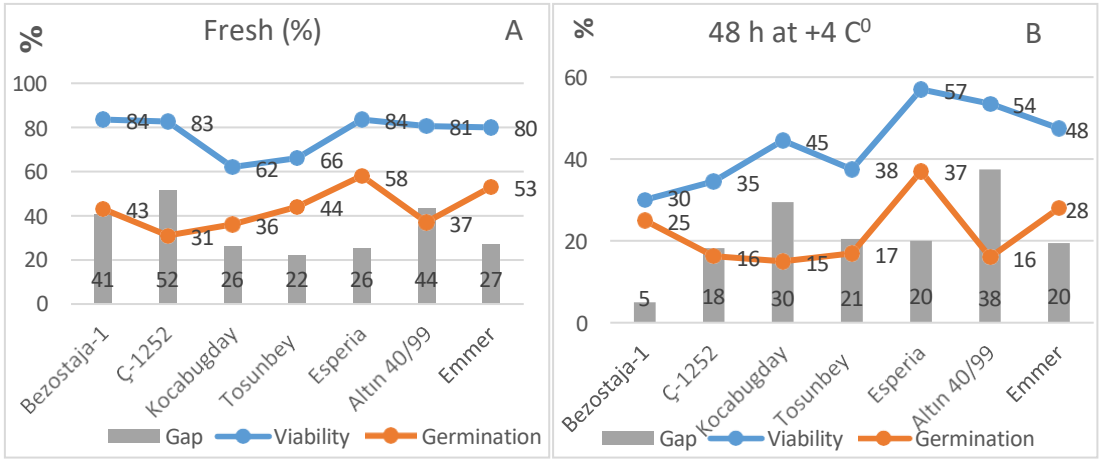

Figure 5. Gap between pollen viability and germination in fresh (A) and 48 h stored (B)

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Discussion

Pollen viability is of great interest to breeders and researchers alike. Breeding projects may wish to store pollen for extended periods of time, where high value genetic material may be stored for future use or for biotechnological and gene editing applications that requires a quick and effective method for determining pollen viability. Different staining (dyes) and impedance flow cytometry (IFC) methods are used in pollen viability tests. The results of the two methods are similar (Kanwal et. al., 2022). In our study using the tetrazolium test (TTC), the average fresh pollen viability of the varieties was found to be 77%. In previous studies, the results varied widely. Kanwal et al. (2022) 70-100%, and Impe et al. (2020) 79% reported viability. The wide variation in fresh pollen viability is due to many factors such as variety, developmental nature of the variety, test method used, test duration, pollen harvest, temperature and water stress, and ambient humidity (Asseng et al., 2011; Parrotta et al., 2016; Kanwal et al., 2022; Khan et. al., 2022). The capacity of pollen to remain viable after being preserved for an extended period of time is known as its longevity. Wheat pollen can remain viable for 5 h at 20 ^{o}C (Kanwal et al., 2022). When the anthers start to open, the average humidity is around 30-35% and from this moment on, the pollen starts to dry rapidly and lose its viability (Jayaprakash et al., 2015). Therefore, pollen viability decreases rapidly over time in storage environments. Loss of viability is clearly seen in the results of our study and similar studies (Impe et al., 2020; Kanwal et al., 2022;).

In our study, the average fresh germination of the varieties in the *in vitro* germination tests was found to be 43%. Different results were obtained in previous studies. Baninasab et al. (2017) stated that it varied between 5-84% and the average of the varieties was 50%, Kanwal et al. (2022) found 66-91%, and Bheemanahalli et al. (2019) found an average of 60%.

Depending on the time elapsed after pollen harvest, the germination of stored pollen decreases rapidly. However, Kanwal et al. (2022) reported that germination decreased by

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10% (from 80% to 70%) after 5 h in an *in vitro* environment. Nonetheless, there aren't many research that concentrate on the short-term storage of spring wheat pollen at different temperatures. In order to maintain pollen viability, proper storage temperatures are essential. Therefore, our study's main goals were to identify the ideal temperature range for keeping spring wheat pollen and the amount of time that wheat pollen can be kept in a variety of settings without losing its viability (Mosquera, 2021). In our study, germination decreased by half because of storage at +4 ^{o}C for 48 h. In storage at room temperature and -18 ^{o}C, germination rates decreased to 0. Baninasab et. al. (2017) reported that the germination decreased to 0 at room temperature and -20 ^{o}C in 24 h and the germination was 1-16% at +4 ^{o}C. It has been reported that temperatures below 0 ^{o}C can lead to ice formation inside the cell, which can lead to cell death and germination losses (Bhat et al., 2012). According to Baninasab et al. (2017), Khan and Sajjad (2023), and the results of our study, the optimum wheat pollen storage temperature was determined as 2-5 ^{o}C without different treatments. Temperature is more effective than humidity in maintaining viability (Impe et. al., 2019). In addition, the germination (2-14%) period can be prolonged up to 1 week when pollen is stored with the spike at 4 ^{o}C (Khan and Sajjad, 2023).

One of the important issues in pollen germination tests is to ensure that the time between pollen collection and germination trials is short and that the pollen is stored in a protected manner during this period. Temperatures at the time of pollen collection and the time of collection are effective for pollen germination. An increase in daytime temperature from 24 to 34 ^{o}C can reduce pollen germination by 2-93% (Bheemanahalli et al., 2019). High temperature damages the physical structure, composition, or energy (sugar) status of pollen (Jagadish et al., 2010; Jiang et al., 2015; Djanaguiraman et al., 2017; Prasad et al., 2017; Sita et al., 2017). This may result in a decrease in the grain set.

Pollen viability and germination tests give different results from those in previous studies. Although pollen viability is high, germination rates are low. Some pollen that has lost their germination properties but appear viable in the staining (viability tests) tests. This is clearly seen in the results of our study. The difference is proportionally similar in many varieties. Germination rates of the varieties with high viability ratios were also high.

In our study, the viability and germination rates of fresh and stored pollen varied among varieties, as reported by Kanwal et al. (2022). Features such as flower physiology, temperature, moisture content of pollen, and developmental stage, plant height, filament length, winter or spring wheat variety are effective on the viability of pollen, especially species and variety characteristics (Koubouris et al., 2002; Patel and Mankad, 2014; Liu et al., 2023). In a study comparing the difference between winter and spring varieties, it was reported that spring varieties showed higher germination characteristics in an *in vitro* environment (Bheemanahalli et al., 2019; Impe et al., 2020). While the variation was much wider in spring varieties and the average vigor was low, the vigor was found to be more homogeneous and higher in winter varieties (Kanwal et al., 2022). While the vigor was found to be very high in some of the local wheat varieties, this was not the case for all local varieties (Ugarte et al., 2007; Asseng et al., 2011).

In studies on the sugars (maltose, sucrose, fructose, galactose, dextrose, lactose, raffinose) that should be present in *in vitro* pollen germination media, it is stated that different sugars and doses give better results for different plant pollen. The appropriate germination medium for wheat pollen should be 0.81 mM H_3BO_3, 2.04 mM $CaCl_2$-$2H_2O$, and 594 mM raffinose, pH 5.8 (Impe et al., 2020). Impe et al. (2020) stated that raffinose (594 mM and 0.75 M doses) is the most suitable sugar for wheat pollen germination and sucrose decreases pollen germination. However, high germination rates were obtained by using Sucrose in Kanwal et. al. (2022) and in the results of our study. It was reported that Sucrose and Maltose can replace Raffinos (Cook and Walden, 1965).

Conclusions

Wheat pollen, regardless of the storage temperature, lost their germination properties after 48 hours, although they appeared to be alive in staining tests. The optimum storage temperature was determined as 4 degrees Celsius. In fresh and post-storage tests, not all of the pollen that appear viable in staining tests can germinate in vitro. In other words, only 2/3 of the stained pollen germinates. The germination rates of the varieties with high vigour were also found to be high.

Authors' Contributions

BA conceived the study, the statistical analysis, prepare the manuscript ; SFG did germination tests. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Asseng, S., Foster, I., and Turner N.C. 2011. The impact of temperature variability on wheat yields. Glob Chang Biol. 17:997–1012. doi:10. 1111/j.1365-2486.2010. 02262.x

Baninasab, B., Tabori M., Yu, J., Zhang, Y., Wang, X., Deschiffart, I., and Khanizadeh, S. 2017. Low temperature storage and in-vitro pollen germination of selected spring wheat accessions. Journal of Agricultural Science 9(9):1-6.

Bhat, Z.A., Dhillon, W.S., Shafi, R.H.S., Rather, J.A., Mir, A.H., Shafi, W., et al. 2012. Influence of storage temperature on viability and *in vitro g*ermination capacity of pear (*Pyrus* spp.) pollen. Journal of Agricultural Science 4:128-135. https://doi.org/10.5539/jas.v4n11p128

Bheemanahalli, R., Sunoj, V.J., Saripalli, G., Prasad, P.V., Balyan, H.S., Gupta, P.K., and Jagadish, S.K. 2019. Quantifying the impact of heat stress on pollen germination, seed set, and grain filling in spring wheat. Crop Science 59(2):684-696.

Choudhry, N.; Siddiqui, M.; Bi, S.; Khatoon, S. Effect of seasonality and time after anthesis on the viability and longevity ofCannabis sativapollen. Palynology 2014, 38, 235–241

Cook, F.S., and Walden, D.B. 1965. The male gametophyte of *Zea mays* L.: II. *İn vitro* germination. Canadian Journal of Botany 43(7):779-786.

De Storme, N., and Geelen, D. 2014. The impact of environmental stress on male reproductive development in plants: biological processes and molecular mechanisms. Plant Cell Environ. 37, 1–18. doi: 10.1111/pce.12142.

Djanaguiraman, M., Perumal, R., Ciampitti, I.A., Gupta, S.K., and Prasad, P.V.V. 2017. Quantifying pearl millet response to high temperature stress: Thresholds, sensitive stages, genetic variability and relative sensitivity of pollen and pistil. Plant Cell Environ. 41:993–1007. doi:10.1111/pce.12931

Güçlü, S.F., and Koyuncu, F. 2017. Effects of relative humidity on *in vitro* pollen germination and tube growth in sweet cherries (Prunus Avium L.). Scientific Papers Series B. Horticulture 61:15-20.

ISSN: 0028-0712

Güçlü, S.F., Sarıkaya, A.G., and Koyuncu, F. 2018. Pollen Performances of Naturally Grown Blackberries in Isparta-Turkey. Scientific Papers Series B. Horticulture 62:141-146.

Güçlü, S. F., Öncü, Z., & Koyuncu, F. (2020). Pollen performance modelling with an artificial neural network on commercial stone fruit cultivars. *Horticulture, Environment, and Biotechnology*, *61*(1), 61-67.

He, W.; Xiao, Q.; Pu, G.; Huang, X.; Li, Y.; Shi, L. 2017. Effect of walnut pollen on 'Shuangzao' fruit quality and early fruit of several. J. Hunan Agri. Univ., 43, 266–269

Impe, D. 2022. Wheat pollen viability and physiological, biochemical and biophysical factors impacting pollen storability. PhD Tesis. Faculty of Natural Sciences, Gottfried Wilhelm Leibniz University, Hannover, Germany.

Impe, D., Ballesteros, D., Ischebeck, T., Köpnick, C., Rolletschek, H., Melzer, M., and Nagel, M.2019. Towards Long-Term Storage of Wheat Pollen. Cryobiology 91:161

Impe, D., Reitz, J., Köpnick, C., Rolletschek, H., Börner, A., Senula, A., and Nagel, M. 2020. Assessment of pollen viability for wheat. Frontiers in Plant Science 10:1588.

Jagadish, S.V.K., Muthurajan, R., Oane, R., Wheeler, T.R., Heuer, S. Bennett, J., and Craufurd, P.Q. 2010. Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). J. Exp. Bot. 61(1):143–156. doi:10.1093/jxb/erp289

Jayaprakash, P., Annapoorani, S., Vikas, V.K., Sivasamy, M., Kumar, J., Saravannan, K., Sheeba, D. 2015. An improved in-vitro germination medium for recalcitrant bread wheat (*Triticum aestivum* L.) pollen. Indian J. Genet. Plant Breed. 75:446–452.

Jia, H.; Liang, X.; Zhang, L.; Zhang, J.; Sapey, E.; Liu, X.; Sun, Y.; Sun, S.; Yan, H.; Lu, W.; et al. Improving ultra-low temperature preservation technologies of soybean pollen for off-season and off-site hybridization. Front. Plant Sci. 2022.

Jiang, Y., Lahlali, R., Karunakaran, C., Kumar, S., Davis, A.R., and Bueckert, R.A. 2015. Seed set, pollen morphology and pollen surface composition response to heat stress in field pea. Plant Cell Environ. 38:2387–2397. doi:10.1111/ pce.12589

Jumrani, K.; Bhatia, V.S.; Pandey, G.P. 2018. Screening soybean genotypes for high temperature tolerance by in vitro pollen germination, pollen tube length reproductive efficiency and seed yield. Indian J. Plant Physiol. 23, 77–90.

Kadri, K., Elsafy, M., Makhlouf, S., & Awad, M. A. (2022). Effect of pollination time, the hour of daytime, pollen storage temperature and duration on pollen viability, germinability, and fruit set of date palm (Phoenix dactylifera L.) cv" Deglet Nour". *Saudi Journal of Biological Sciences*, *29*(2), 1085-1091.

ISSN: 0028-0712

Kanwal, M., Gogoi, N., Jones, B., Bariana, H., Bansal, U., and Ahmad, N. 2022. Pollen: A Potential Explant for Genetic Transformation in Wheat (*Triticum aestivum* L.). Agronomy 12(9):2-15.

Khan, I., and Sajjad, M. 2023. A simple method for preserving pollen viability and longevity in wheat. https://doi.org/10.21203/rs.3.rs-2580944/v1

Khan, I., Wu J., and Sajjad, M. 2022. Pollen viability-based heat susceptibility index (HSIpv): A useful selection criterion for heat-tolerant genotypes in wheat. Frontiers in Plant Science 13:01-11. DOI 10.3389/fpls.2022.1064569

Khan, I., Naeem, M. K., Shahzad, A., Zhang, Z., Chen, J., & Sajjad, M. (2024). Optimizing wheat pollen preservation for enhanced viability and in vitro germination. *Agronomy*, *14*(1), 201.

Koubouris, G.C., Metzidakis, I.T., and Vasilakakis, M.D. 2009. Impact of temperature on olive (Olea europaea L.) pollen performance in relation to relative humidity and genotype. Environ. Exp. Bot. 67:209–214

Liu, X., Xiao, Y., Zi, J., Yan, J., Li, C., Du, C., Wan, J., Wu, H., Zheng, B., Wang, S., et al. 2023. Differential effects of low and high temperature stress on pollen germination and tube length of mango (Mangifera indica L.) genotypes. Sci. Rep. 13(1): 611.

Longin, C.F.H., and Reif, J.C. 2014. Redesigning the exploitation of wheat genetic resources. Trends in plant science, 19(10):631-636. https://doi.org/10.1016/j.envexpbot.2009.06.002

Masthigowda, H.M., Sharma, D., Khobra, R., Krishnappa, G., Khan, H., Singh, S.K., et al. 2022. Pollen viability as a potential trait for screening heat-tolerantwheat (Triticum aestivum l.). Funct. Plant Biol. 49(7):625–633. doi:10.1071/FP21096

McCormick, S. 2004. Control of male gametophyte development. Plant Cell, 16, S142–S153.

Mosquera, D.J.C.; Salinas, D.G.C.; Moreno, G.A.L. 2021. Pollen viability and germination in Elaeis oleifera, Elaeis guineensis and their interspecific hybrid. Pesqui. Agropecuária Trop., 51, e68076.

Mukti, G. 2014. Pollen storage and viability. Int. J. Bot. Res. 4, 1–18

Parrotta, L., Faleri, C., Cresti, M., and Cai, G. 2016. Heat stress affects the cytoskeleton and the delivery of sucrose synthase in tobacco pollen tubes. Planta 243:43–63.

Patel, R.G., and Mankad, A.U. 2014. In vitro pollen germination-A review. Int. J. Sci. Res. 3:304–307.

Prasad P.V.V., and Djanaguiraman, M. 2014. Response of floret fertility and individual grain weight of wheat to high temperature stress: sensitive stages and thresholds for temperature and duration. Functional Plant Biology 41, 1261–1269.

ISSN: 0028-0712

Prasad, P.V.V., Bheemanahalli, R., and Jagadish, S.V.K. 2017. Field crops and the fear of heat stress: Opportunities, challenges and future directions. Field Crops Res. 200:114–121. doi:10.1016/j.fcr.2016.09.024

Resende, K., Prado, C., Davide, L., and Torres, G. 2014. Polyploidy and apomixis in accessions of Senna rugosa (G. Don) HS Irwin & Barneby. Turkish Journal of Biology 38(4):510-515.

Sita, K., Sehgal, A., Kumar, J., Kumar, S., Singh, S., Siddique, K.H., and Nayyar, H. 2017. Identification of high-temperature tolerant lentil (*Lens culinaris* Medik.) genotypes through leaf and pollen traits. Front. Plant Sci. 8:744. doi:10.3389/fpls.2017.00744

Ugarte, C., Calderini, D.F., and Slafer, G.A. 2007. Grain weight and grain number responsiveness to pre-anthesis temperature in wheat, barley and triticale. Field Crops Res. 100:240–248. doi:10.1016/j.fcr.2006.07.010