Anatolian Journal of Botany
7(1): 70-75 (2023)
Research article
doi: 10.30616/ajb.1254465

Pollen performance of pomegranate under high-temperature stress

Melse Su BİLGİLİ[1], **Aslıhan ÇETİNBAŞ GENÇ**[2]*
[1]*MarmaraUniversity, Science Faculty, Department of Biology, İstanbul, Türkiye*
**aslihan.cetinbas@marmara.edu.tr, [1]melsesubilgili@gmail.com*

Received : 21.02.2023
Accepted : 07.04.2023
Online : 13.04.2023

Yüksek sıcaklık stresi altında narın polen performansı

Abstract: The high-temperature stress (30 °C, 35 °C, 40 °C) response of pollen performance in *Punica granatum* was analyzed. Pollen germination rate and tube length were significantly inhibited after 35 °C and 40 °C treatment. According to cumulative stress response index values, 40 °C had the most destructive impact. High-temperature stress caused various abnormalities at tubes, especially at apex and the most common abnormalities were marked change of elongation direction and swelling. Although dense callose accumulation and increase in apex-localized reactive oxygen species was noticed at the apex after 35 °C and 40 °C temperature treatment, the most harmful temperature was stated as 40 °C.

Key words: High-temperature, pollen germination, pollen tube, *Punica granatum*

Özet: Bu çalışmada *Punica granatum*'da polen performansının yüksek sıcaklık stresine (30 °C, 35 °C, 40 °C) olan tepkisi analiz edildi. Polen çimlenme oranı ve tüp uzunluğu 35 °C ve 40 °C uygulamasından sonra istatistiksel olarak anlamlı bir şekilde azaldı. Kümülatif stres tepki indeksi değerlerine göre, 40 °C polen performansı üzerinde en yıkıcı etkiye sahipti. Yüksek sıcaklık stresi polen tüplerinin özellikle uçlarında çeşitli anormalliklere neden oldu. En yaygın görülen anormallikler uzama yönünün değişmesi ve tüp uçlarının şişmesiydi. 35 °C ve 40 °C uygulamalarından sonra tüp uçlarında yoğun kalloz birikimi ve reaktif oksijen türlerinde artış görülmesine rağmen, en yıkıcı yüksek sıcaklık stresi 40 °C olarak belirlendi.

Anahtar Kelimeler: polen çimlenmesi, polen tüpü, *Punica granatum*, yüksek sıcaklık

Citation: Bilgili MS, Çetinbaş Genç A (2023). Pollen performance of pomegranate under high-temperature stress. Anatolian Journal of Botany 7(1): 70-75.

1. Introduction

Punica granatum L. (pomegranate), a member of the *Punicaceae* family, is one of the oldest plants in the world which has commercial and cultural importance. Since it is a prominent crude ingredient for the pharmaceutical, cosmetic, beverage, and food industries, the economic value of pomegranate fruits is very high (Khemakhem et al., 2021). Pomegranate fruits are frequently preferred in people's daily diets due to its vitamin, mineral, phenols, flavonoids, and antioxidant content (Boussaa et al., 2020). Also, demand for pomegranate fruit has been increasing rapidly, especially in recent years, after the protective impacts of pomegranate against Alzheimer's disease and cancer were revealed in all details (Teniente et al., 2023). Parallel with increasing demand, the definition of the agents influencing the yield of pomegranate and the studies to increase the yield has accelerated, and these topics have started to attract more attention from researchers (Moga et al., 2021). Reproductive biology is the most important features that needs to be studied while carrying out studies about these topics in such an important medical and economic plant.

Pomegranate has perfect flowers and functional staminate flowers on the same tree. Staminate flowers are small and don’t form fruits due to the they have rudimentary ovary. However, they are important and necessary for successful pollination due to they produce a large number of fertile pollen grains. Perfect flowers are large and, have functional ovary which forms fruit (Wetzstein et al., 2011). Although they also produce pollen grains, pollen viability of perfect flowers is largely lower than pollen viability of staminate flowers (Engin and Hepaksoy, 2023). Besides, pomegranate shows protogynous dichogamy that pistil gains reproductive activity much before the anther dehisces (Morbey and Ydenberg, 2001; Engin and Hepaksoy; 2003). When the pistil of the hermaphrodite flower gains reproductive activity, anthers of the hermaphrodite flower have not yet begun to pollen shedding (Çetinbaş-Genç and Ünal, 2017). So, the pistil of the hermaphrodite flower is mostly pollinated and fertilized by pollen grains of the male flowers. In this way, fertilization and therefore fruit formation mostly depend on pollen performance (PP) of male flowers in pomegranate (Engin and Hepaksoy, 2003). For this reason, it is very important to know the impacts of various factors that adversely affect PP while carrying out studies to increase yield in pomegranate because it could enable the producer to take precautions against these factors.

PP can be monitored by various parameters. The first parameter to be used to examine PP is pollen germination (PG), because PG is the first step pollen tube (PT) formation, which will ensure the move of generative nucleus to the embryo sac (ES) in the latter stages (Cascallares et al., 2020). Since the PT should be sufficiently lengthy to arrive the ES, it is also very important to evaluate the PT length when examining PP. Also, the cumulative stress response index (CSRI) computed using PG rate and PT length can also give an idea about PP (Dai et al., 1994). PT enter the ES through the micropylar gap of the ovule, and the diameter of this gap is generally proportional to the PT diameter. A morphological

---

abnormality in the PT may prevent the PT entering the ES via the micropyle opening. Therefore, it is very important for the PTs to maintain their unique dome-tipped cylinder shape during the PT elongation process for successful fertilization, and abnormalities seen in the PTs are considered a negative condition in PP evaluations (Srinivasan et al., 1999). Moreover, alterations in the unique construction of the PT wall mainly consisting of callose and cellulose (also pectin) are also used to evaluate PP. Cellulose is found throughout the PT including the apex, while callose is found along the PT except for the apex (Hao et al., 2013). The absence of callose at the apical region is necessary for the PT elongation (Wang et al., 2003). That's why the accumulation of callose at the PT apex is considered a low PP. Also, tip-localized ROS is essential to ensure the proper PT elongation and regulate the stress responses (Scholz et al., 2020). Due to the tip-localized ROS is over accumulate under abiotic stress conditions, changes in tip-localized ROS accumulation can be utilized as a mark to measure the damage in PP under stress conditions (You and Chan, 2015).

High-temperature stress (HTS) is a restraining agent for both the vegetative and generative growth of plants. Especially the HTS exposure during the sexual reproduction process causes significant losses in product productivity (Ferguson et al., 2021). Although it is known that gametophytes are susceptible to temperature changes, pollen grains, which are male gametophytes, are more sensitive to temperature changes as they have an active interaction with the environment. Sudden temperature changes or extreme day-night temperature differences caused by global warming reduce the PP of the species and cause a decrease in yield (Zhu et al., 2021). That's why PP is mostly used as an indicator for the determination of impacts generated by temperature change (Mesihovic et al., 2016). In many studies, the impacts of HTS on the evaluation factors mentioned above have been examined separately. For example, HTS has been shown to reduce PG and PT elongation in many species such as groundnut (Kakani et al., 2002), olive (Koubouris et al., 2009), tobacco (Parrotta et al., 2016) and almond (Sorkheh et al., 2018). Also, it has been determined that HTS causes changes in tube morphology in various species such as *Arabidopsis thaliana* (Boavida and McCormick, 2007) and tea plants (Wang et al., 2016). Also, it has been noticed that HTS changes the ROS accumulation and cell wall properties in PTs of tomatoes (Muhlemann et al., 2018). However, the number of studies in which a general evaluation is made by using all parameters is very few. Many researchers have conducted various studies examining the quality of pomegranate pollen (Gadze et al., 2011; Engin and Gökbayrak, 2016) and have carried out various studies to increase PP with various plant growth regulators (Gökbayrak and Engin, 2018; Korkmaz and Güneri, 2019). However, there are no studies indicating the impacts of HTS on pomegranate pollen grains.

The main objective of this study to investigate the PP of pomegranate under HTS, using the PP evaluation factors such as germination rate, PT length, PT morphology, callose accumulation, tip-localized ROS accumulation. The results can provide useful information to increase PP, fertilization success, and fruit set for plants grown in heat-prone regions, especially pomegranate.

2. Materials and Method

Staminate flowers were selected in 2019 spring from three different healthy trees of *Punica granatum* L. located in Akçakoca/Düzce (Türkiye). No chemical was applied to the trees since 2014 summer. Collected pollen grains were germinated at 30 °C, 35 °C and 40 °C for 3 h in PG media with 12% sucrose (Brewbaker and Kwack, 1963; Korkmaz and Güneri, 2019). Germinated pollen grains at 25 °C were used as control. PG rates, PT lengtHTS and PT abnormality rates were calculated by counting 150 pollen grains for each group. Observations were made by a light microscope. CSRI was computed to appreciate the HTS response of PTs using PG rates and PT lengtHTS of control and treatment groups with Dai's equation (Dai et al., 1994). PTs were labelled with 0.1% aniline blue for callose accumulation and with 5-(and 6-)chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) for tip-localized ROS, and investigated respectively at 455-nm wavelengtHTS and 500-nm with an Olympus BX-51 fluorescence microscope (Chen et al., 2007). Fluorescence intensities (FIs) of aniline blue and H_2DCFDA were calculated in a 100-μm^2 zone of the 60 tube tip by the "Rectangle Selection" option of ImageJ. Statistical examinations were executed with the SPSS 16.0 software. The significance of the difference between groups of data was defined by the one-way analysis of variance (ANOVA) with a threshold P value of 0.05. Different characters in graps specify the statistically significant differences and error bars show the standard deviations.

3. Results

To observe the preliminary impact of HTS on PP, PG rate and PT length were calculated. Based on the results, no statistically significant alterations were monitored in PG rate and PT length at 30 °C treatment when compared with the control. Nevertheless, the PG was significantly decreased by 15.62% at 35 °C and 32.44% at 40 °C when compared control. Besides, PT length was significantly decreased by 12.16% at 35 °C and 22.7% at 40 °C in comparison with the control (Figs. 1a,b). The CSRI score of HTS treated groups was reckoned using the PG and PT length. According to the CSRI score, all groups showed negative data, indicating all high-temperature treatments had a minus impact on PP. CSRI score was -2.96 for 30 °C, -27.28 for 35 °C and -55.22 for 40 °C. As the CSRI scores show, the minimum detrimental impact was determined at 30 °C while the maximal detrimental impact was identified at 40 °C. To distinguish between the impact of HTS treatments on tube morphology, PT abnormalities were examined and it was determined that HTS caused various abnormalities at PTs, especially at apex. The most common abnormalities were sharp change of elongation direction and swelling (Fig. 1c). PT abnormality rate was significantly increased by nearly 2-fold at 30 °C and 35 °C while it was significantly increased by nearly 3-fold at 40 °C (Fig. 1d).

To examine the impact of HTS on the callose distribution of PT wall, PTs were stained by aniline blue. According to aniline blue staining, callose was localized throughout the PT except apex at control and 30 °C. However, intensive callose accumulation was noticed at the apex after 35 °C and 40 °C treatment (Fig. 2a). FI of callose was significantly increased by 59.31% at 35 °C and 196.58% at 40 °C when compared control (Fig. 2b). To investigate the impact of HTS on the tip-localized ROS accumulation, PTs were labelled by H_2DCFDA. According to results, although PTs of all groups demonstrated an obviously tip-localized ROS, the ROS was more intense especially after 35 °C and 40 °C treatment, when compared with the control (Fig. 2c). FI of ROS was significantly increased by 24.77% at 35 °C and 43.79% at 40 °C when compared control (Fig. 2d).

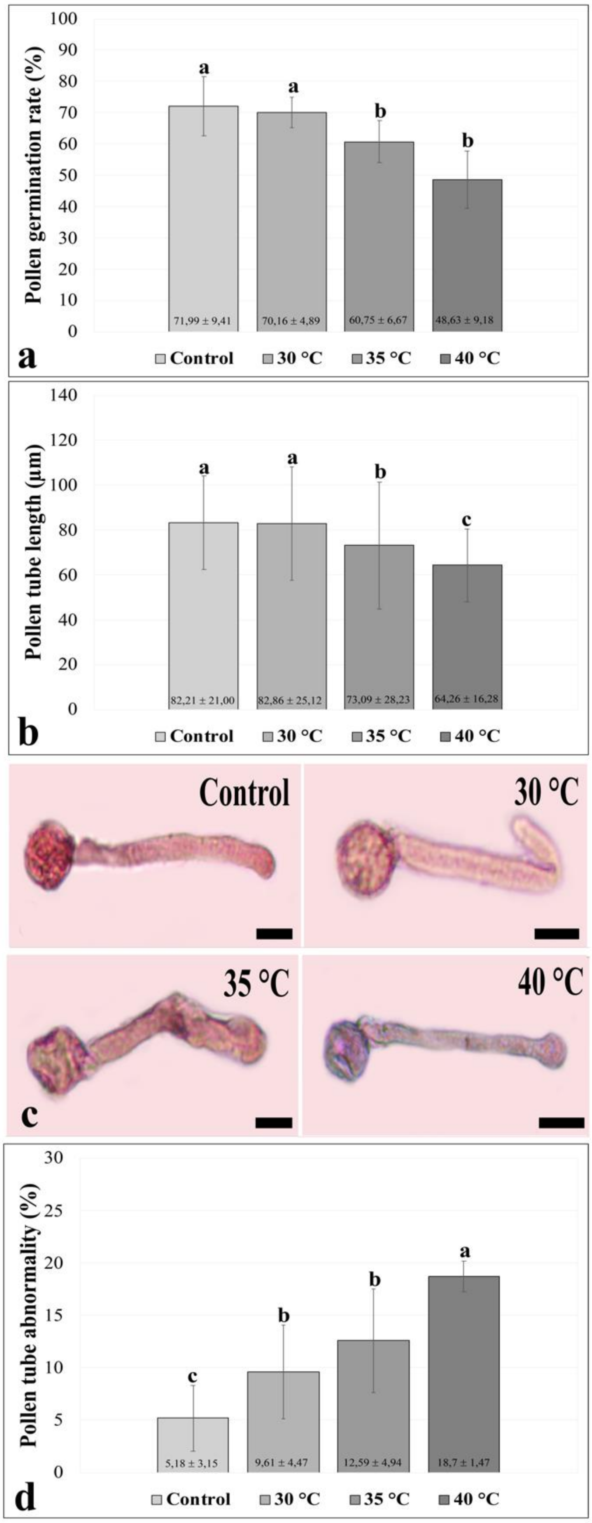

Figure 1. The impact of HTS on PG, PT length and PT morphology in *P. granatum*. **a.** PG rate, **b.** PT length, **c.** Exemplary images of PT abnormalities, **d.** PT abnormality rate. Bar: 10 µm.

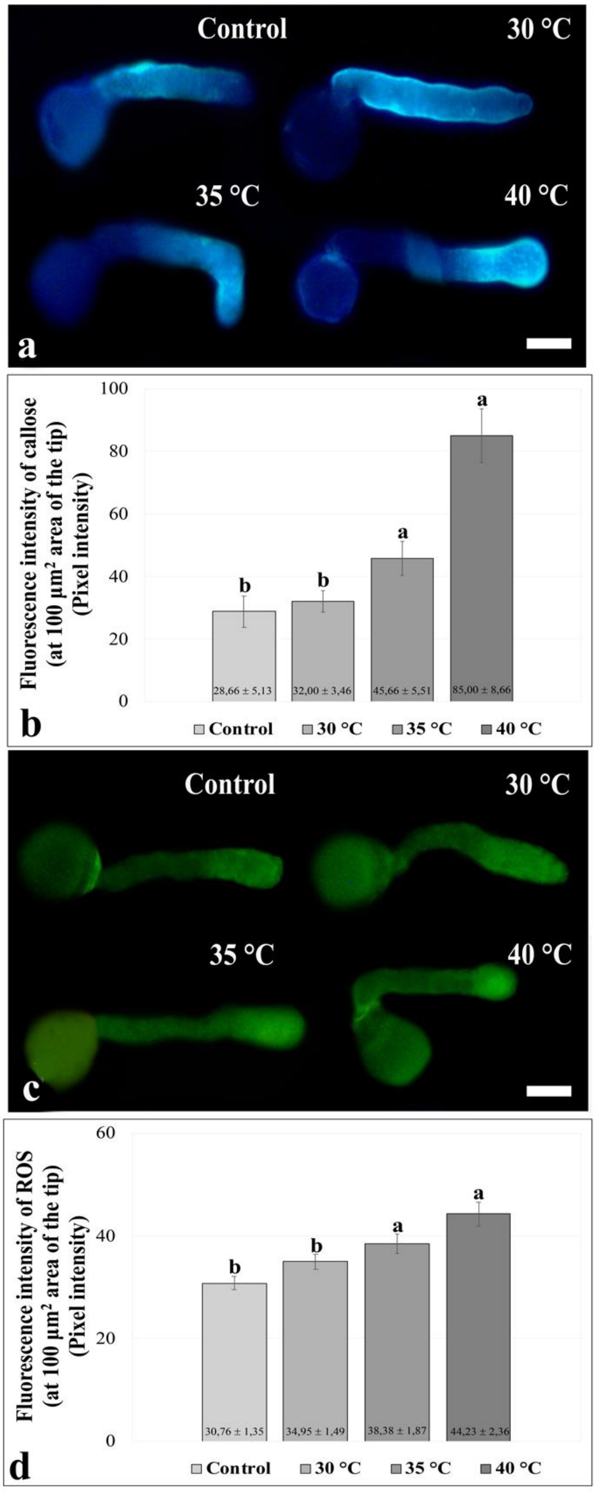

Figure 2. Impact of HTS on callose distribution, tip-localized ROS accumulation and ROS detoxification system in *P. granatum*. **a.** Exemplary images of callose accumulation, **b.** FI of callose accumulations, **c.** Exemplary images of tip-localized ROS accumulation, **d.** FI of tip-localized ROS accumulations. Bar: 10 µm.

4. Discussions

This section should underly the meaning of your research, or highlight the importance of your study and how it may be able to contribute to and/or help fill existing gaps in the field.

It has been known that low or high temperature mostly have opposite impacts on PP (Krawczyk et al., 2022). For instance; PG and PT length were decreased by high-temperature treatment at 30 °C in hazelnut (Çetinbaş-Genç et al., 2019) and, decreased by high-temperature treatment above 35 °C in cotton and tobacco (Song et al., 2015; Parrotta et al., 2016). In pomegranate, PG rate and PT length were remarkably inhibited after 35 °C and 40 °C treatment while 30 °C did not make any significant change. To reveal the most devastating temperature on PP, CSRI values of treatment groups were calculated. CSRI values showed that although all treatment temperatures had an unfavorable effect on PP, 40 °C had the most devastating effect with high negative value. Negative CSRI datas also have been stated in PTs of almond that exposed to 30 °C and 40 °C (Sorkheh et al., 2018) and in PTs of hazelnut that subjected to 30 °C (Çetinbaş-Genç et al., 2019).

Angiosperm PTs are generally linear structures with a dome-shaped tip. It has been known that various temperature stress can alter this PT morphology (Srinivasan et al., 1999). Distribution of PT morphology in angiosperm PTs, especially at apex, usually means that the growth is inhibited. (Wang et al., 2016) have been stated the swelling of PT in cold inhibited PTs of tea. Moreover, (Çetinbaş-Genç et al., 2019) have been noticed that low and high temperature obviously disrupts the morphology of PT tips in hazelnut. Parallelly with these literatures, it was detected that HTS caused various abnormalities at PTs after all treatment groups while the most increase in PT abnormality was detected after 40 °C.

Temperature stress can also alter the PT wall properties, especially accumulation of callose (Parrotta et al., 2019). In angiosperm PTs, callose is abundantly localized compound along the PT except the apex (Qin et al., 2012). Moreover, callose accumulation at the apex is considered a decrease in PP because callose accumulation at apex prevents the transfer of sperm nuclei, reducing fertilization success (Wang et al., 2003). Based on our findings, callose at PT tips increased after 30 °C and 40 °C treatment. Unconventional callose accumulation is produced by modifications in actin structure and organization (Parrotta et al., 2022). Corruption of actin cytoskeleton obstructs the generation of functional PT by blocking PG and PT growth (Cai et al., 2011). And also, it has been known that various stress conditions, especially the temperature stress cause callose deposition on the PT tip (Kapoor and Geitmann, 2023). So, it can be hypothesized that 30 °C and 40 °C temperature disrupts the actin skeletal structure, both preventing PT elongation and causing callose accumulation at the PT tips.

Tip-localized ROS is requise to ensure PT elongation (Swanson and Gilroy, 2010). Due to the tip-localized ROS is over accumulate under abiotic stress cases, alterations in tip-localized ROS accumulation can be utilized as a mark to measure the harmfull effects in PP under stress conditions (Aloisi et al., 2022). Wang et al. (2016) and Parrotta et al. (2019) noticed that cold stress improved the ROS signal in the PT apex of *Camellia sinensis* (L.) Kuntze and *Nicotiana tabacum* L., respectively. Parallelly with those results, we observed that the apex-localized ROS accumulation enhanced after 30 °C and 40 °C treatment. It has been known that regular accumulation of ROS at the PT apex directly or indirectly regulates the actin filament organization (Zonia, 2010). So, it can be hypothesized that 30 °C and 40 °C temperature increase the tip-localized ROS disrupting actin skeletal structure, both preventing PT elongation and causing callose accumulation at the PT tips.

5. Conclusion

High-temperature stress is negatively affected the PP of pomegranate. PG rate and PT length are decreased and, PT abnormality, callose accumulation at apex and tip-localized ROS accumulation are increased after 35 °C and 40 °C high-temperature treatment. These findings show that HTS may prevent the pollination and fertilization processes in pomegranate.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Aloisi I, Piccini C, Cai G, Del Duca S (2022). Male fertility under environmental stress: Do polyamines act as pollen tube growth protectants?. International Journal of Molecular Sciences 23(3): 1874.

Boavida LC, McCormick S (2007). Technical advance: Temperature as a determinant factor for increased and reproducible in vitro pollen germination in *Arabidopsis thaliana*. The Plant Journal 52(3): 570-582.

Boussaa F, Zaouay F, Burlo-Carbonell F, Noguera-Artiaga L, Carbonell-Barrachina A, Melgarejo P, Hernandez F, Mars M (2020). Growing location affects physical properties, bioactive compounds, and antioxidant activity of pomegranate fruit (*Punica granatum* L. var. *gabsi*). International Journal of Fruit Science 20: 508-523.

Brewbaker JL, Kwack BH (1963). The essential role of calcium ion in pollen germination and pollen tube growth. American Journal of Botany 50(9): 859-865.

Cai G, Faleri C, Del Casino C, Emons AMC, Cresti M (2011). Distribution of callose synthase, cellulose synthase, and sucrose synthase in tobacco pollen tube is controlled in dissimilar ways by actin filaments and microtubules. Plant Physiol 155(3): 1169-1190.

Cascallares M, Setzes N, Marchetti F, López GA, Distéfano AM, Cainzos M, Zabaleta E, Pagnussat GC (2020). A Complex journey: cell wall remodeling, ınteractions, and ıntegrity during pollen tube growth. Frontiers in Plant Science 11: 599247.

Chen T, Teng N, Wu X, Wang Y, Tang W, Samaj J, Baluska F, Lin J (2007). Disruption of actin filaments by latrunculin B affects cell wall construction in *Picea meyeri* pollen tube by disturbing vesicle trafficking. Plant Cell Physiology 48: 19-30.

Çetinbaş-Genç A, Ünal M (2017) Timing of reproductive organs maturity in proterandrous *Malva sylvestris* L. Notulae Scientia Biologicae 9(2): 287-295.

Çetinbaş-Genç A, Cai G, Vardar F, Ünal M (2019). Differential effects of low and high temperature stress on pollen germination and tube length of hazelnut (*Corylus avellana* L.) genotypes. Scientia Horticulturae 255: 61-69.

Dai Q, Shaobing P, Chavez AQ, Vergara BS (1994). Intraspecific responses of 188 rice cultivars to enhanced UVB radiation. Environmental and Experimental Botany 34(4): 433-442.

Engin H, Gökbayrak Z (2016). In vitro pollen viability and germination of bisexual and functional male flowers of some Turkish pomegranate cultivars. Agriculture & Forestry 62(4): 91-94.

Engin H, Hepaksoy S (2003). Bazı nar çeşitlerinin çiçek tozu çimlenme güçlerinin belirlenmesi. Ege Üniversitesi Ziraat Fakültesi Dergisi 40(3): 9-16.

Ferguson JN, Tidy AC, Murchie EH, Wilson ZA (2021). The potential of resilient carbon dynamics for stabilizing crop reproductive development and productivity during heat stress. Plant, Cell & Environment 44(7): 2066-2089

Gadže J, Radunic M, Petric IV, Ercisli S (2011). In vitro pollen viability, germination and pollen tube growth in some pomegranate (*Punica granatum* L.) cultivars from Croatia and Bosnia and Herzegovina. Acta Scientiarum Polonorum Hortorum Cultus 10: 297-305.

Gökbayrak Z, Engin H (2018). Effects of foliar-applied brassinosteroid on viability and ın vitro germination of pollen collected from bisexual and functional male flowers of pomegranate. International Journal of Fruit Science 18(2): 226-230.

Hao H, Chen T, Fan L, Li R, Wang X (2013). 2, 6-dichlorobenzonitrile causes multiple effects on pollen tube growth beyond altering cellulose synthesis in *Pinus bungeana* Zucc. PLoS One, 8(10): e76660.

Kakani VG, Prasad PV, Craufurd PQ, Wheeler TR (2002). Response of in vitro pollen germination and pollen tube growth of groundnut (*Arachis hypogaea* L.) genotypes to temperature. Plant Cell and Environmental 25(12): 1651-1661.

Kapoor K, Geitmann A (2023). Pollen tube invasive growth is promoted by callose. Plant Reproduction 36717422 (in print).

Khemakhem M, Zarroug Y, Jabou K, Selmi S, Bouzouita N (2021). Physicochemical characterization of oil, antioxidant potential, and phenolic profile of seeds isolated from Tunisian pomegranate (*Punica granatum* L.) cultivars. Journal of Food Science 86(3): 852-859.

Korkmaz N, Güneri M (2019). Effect of different boron levels on pollen germination of Hicaz nar pomegranate (*Punica granatum* L.) cultivar. International Journal of Agriculture Forestry and Life Sciences 3(1): 151-156.

Koubouris GC, Metzidakis IT, Vasilakakis MD (2009). Impact of temperature on olive (*Olea europaea* L.) pollen performance in relation to relative humidity and genotype. Environmental and Experimental Botany 67(1): 209-214.

Krawczyk HE, Rotsch AH, Herrfurth C, Scholz P, Shomroni O, Salinas-Riester G, Feussner I, Ischebeck T (2022). Heat stress leads to rapid lipid remodeling and transcriptional adaptations in *Nicotiana tabacum* pollen tubes. Plant Physiology 189(2): 490-515.

Mesihovic A, Iannacone R, Firon N, Fragkostefanakis S (2016). Heat stress regimes for the investigation of pollen thermotolerance in crop plants. Plant Reproduction 29: 93-105.

Moga MA, Dimienescu OG, Bălan A, Dima L, Toma SI, Bîgiu NF, Blidaru A (2021). Pharmacological and therapeutic properties of *Punica granatum* phytochemicals: Possible roles in breast cancer. Molecules 26(4): 1054.

Morbey YE, Ydenberg RC (2001). Protandrous arrival timing to breeding areas: a review. Ecology Letters 4(6): 663-673.

Muhlemann JK, Younts TL, Muday GK (2018) Flavonols control pollen tube growth and integrity by regulating ROS homeostasis during high-temperature stress. PNAS 115(47): E11188–E11197.

Parrotta L, Faleri C, Guerriero G, Cai G (2019). Cold stress afects cell wall deposition and growth pattern in tobacco pollen tubes. Plant Sciences 283: 329-342.

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

Parrotta L, Faleri C, Del Casino C, Mareri L, Aloisi I, Guerriero G, Hausman JF, Del Duca S, Cai G (2022). Biochemical and cytological interactions between callose synthase and microtubules in the tobacco pollen tube. Plant Cell Reports 41(5): 1301-1318.

Qin P, Ting D, Shieh A, McCormick S (2012). Callose plug deposition patterns vary in pollen tubes of *Arabidopsis thaliana* ecotypes and tomato species. BMC Plant Biology 12: 178.

Scholz P, Anstatt J, Krawczyk HE, Ischebeck T (2020). Signalling pinpointed to the tip: the complex regulatory network that allows pollen tube growth. Plants 9(9): 1098.

Song G, Wang M, Zeng B, Zhang J, Jiang C, Hu Q, Geng G, Tang C (2015) Anther response to high-temperature stress during development and pollen thermotolerance heterosis as revealed by pollen tube growth and in vitro pollen vigor analysis in upland cotton. Planta 241: 1271-1285.

Sorkheh K, Azimkhani R, Mehri N, Chaleshtori MH, Halasz J, Ercisli S, Koubouris GC (2018). Interactive effects of temperature and genotype on almond (*Prunus dulcis* L.) pollen germination and tube length. Scientia Horticulturae 227: 162-168.

Srinivasan A, Saxena N, Johansen C (1999). Cold tolerance during early reproductive growth of chickpea (*Cicer arietinum* L.): genetic variation in gamete development and function. Field Crops Research 60: 209-222.

Swanson S, Gilroy S (2010). ROS in plant development. Physiologia Plantarum 138(4): 384-392.

Teniente SL, Flores-Gallegos AC, Esparza-González SC, Campos-Múzquiz LG, Nery-Flores SD, Rodríguez-Herrera R (2023). Anticancer effect of pomegranate peel polyphenols against cervical cancer. Antioxidants 12(1): 127.

Wang W, Sheng X, Shu Z, Li D, Pan J, Ye X, Chang P, Li X, Wang Y (2016). Combined cytological and transcriptomic analysis reveals a nitric oxide signaling pathway involved in cold-inhibited *Camellia sinensis* pollen tube growth. Frontiers in Plant Science 7: 456.

Wang Q, Lu L, Wu X, Li Y, Lin J (2003). Boron influences pollen germination and pollen tube growth in *Picea meyeri*. Tree Physiology 23(5): 345-351.

Wetzstein HY, Ravid N, Wilkins E, Martinelli AP (2011). A morphological and histological characterization of bisexual and male flower types in pomegranate. Journal of the American Society for Horticultural Science 136(2): 83-92.

You J, Chan Z (2015). ROS regulation during abiotic stress responses in crop plants. Frontiers in Plant Science 6: 1092.

Zhu T, Fonseca De Lima CF, De Smet I (2021). The heat is on: how crop growth, development, and yield respond to high temperature. Journal of Experimental Botany 72(21): 37359-7373.

Zonia L (2010). Spatial and temporal integration of signalling networks regulating pollen tube growth. Journal of Experimental Botany 61: 1939-1957.