J. Plant Environ. 04 (02) 2022. 155-161 DOI: 10.33687/jpe.004.02.3896

Available Online at EScience Press
Journal of Plant and Environment
ISSN: 2710-1665 (Online), 2710-1657 (Print)
https://esciencepress.net/journals/JPE

Effectiveness of Pollen Germination Media for Improving Storage Potential in Pomegranate (*Punica granatum*) Germplasm

Sufian Ikram[1], Muhammad Jafar Jaskani[1], Salman Ikram[2], Muhammad Ahsan Qureshi[1,4*], Sami-Ur-Rehman[1], Mubasshir Hussain[1], Waqar Shafqat[3], Safeer ud Din[1], Muhammad Shahzad Zafar[4], Samad Raza[4], Syed Inam Ullah Shah Bukhari[4]

[1]*Department of Horticulture, College of Agriculture, University of Sargodha, Pakistan.*
[2]*Department of Horticultural Sciences, Ghazi University, Dera Ghazi Khan-32201, Pakistan.*
[3]*Department of Forestry, Mississippi State University, United States of America.*
[4]*Mango Research Institute, Multan, Pakistan.*

ARTICLE INFO

Article History
Received: July 24, 2022
Revised: September 10, 2022
Accepted: September 15 2022

Keywords
Cryo-preservation
Boron
Invitro germination
Germplasm preservation
Agar-Petri method

ABSTRACT

Viable pollen grains with the ability to germinate after fertilization are necessary for fruit and seed formation. Pollens are sensitive to environmental conditions and quickly lose their viability after anthesis. In vitro storage potential of pollen grains depends on species, genotype, flower type, and storage conditions. The present study's objective was to evaluate pomegranate germplasm's storage potential and to study germination media's influence on short-term stored pollen's germination percentage. Pollens of fifteen pomegranate genotypes were collected and stored at 4 °C for 30 days. Pollen viability was assessed using in vitro pollen germination by the agar-Petri method. The germination media was composed of different concentrations of sucrose, boric acid, and agar. The results showed that germination media (12.5% sucrose + 0.2% agar) supplemented with 10 ppm boric acid gave the highest germination among all cultivars. Among genotypes, maximum (58.3%) germination was observed in Desi and Kandhari red pollens, and minimum (16.3%) was observed in Sandhora. The concentration of boric acid in germination media influenced pollen germination. The highest pollen germination was found at 10 ppm, followed by 5 ppm, and the lowest germination was found in media with no boric acid. Conclusively genotypes show variation in storage potential, and germination media influences post-storage germination rate. The utilization of effective germination media can increase the pollen germination rate for pomegranate genotypes.

Corresponding Author: Muhammad Ahsan Qureshi
Email: ahsanuaf3@gmail.com

INTRODUCTION

Pomegranate belongs to the Punica genus in the Lythraceae family, which is comprised of only two species, with the names of *P. granatum* L. and *P. protopunica* Balf., later is endemic to the Socotra Island of the Arabian Peninsula (Graham *et al*., 2005; Chandra *et al*., 2010; Yuan *et al*., 2018) and is inedible. There are 500 globally distributed varieties, approximately 50 of which are known to be commercially cultivated (Holland *et al*., 2009). The total world production of pomegranate is around 1.5 million tonnes. Global demand for pomegranates is projected to reach USD 208.9 million by 2020 and is projected to reach USD 322.9 million by the

end of 2026, with a compound annual growth rate between 2021 and 2026 of 6.4 percent (Anonymous, 2020). The Pomegranate plant shows remarkable plasticity in growing conditions and can withstand temperatures as high as 45 °C and as low as -10 °C. Considerably tolerant to drought and frost. However, the ideal conditions are hot, dry summer with cool, dry winters (Ikram *et al*., 2020; Mir *et al*., 2012).

Viable pollen grains with the ability to germinate after fertilization are necessary for fruit and seed formation. Pollens are sensitive to environmental conditions and quickly loss viability after anthesis. Invitro storage potential of pollen grains depend upon species, genotype, flower type, and storage conditions (Maryam *et al*., 2015; Astarini, 2019). For pollen viability assessment, several techniques, i.e., staining (Acetocarmine (2%), Tetrazolium (1%), Erythrosin B (0.1%) and in-vitro germination, are used. In-vitro pollen germination is generally more efficient than pollen staining (Gadze *et al*., 2011; Korkmaz and Guneri, 2019). Components of germination media also influence pollen germination rate; adding sucrose and boric acid in agar improves germination depending upon concentration, specie, and genotype (Korkmaz and Guneri, 2019).

Pomegranate has a vast diversity in the time of flowering and fruit set, making it impossible to cross through natural pollination using fresh pollen (Babu, 2010). Storage of pollen is necessary for controlled pollination. Pollen viability differs from cultivar to cultivar. Hence optimization of pollen storage is critical for facilitating breeding programs. So far, no study has been conducted evaluating pollen storage for current pomegranate germplasm. Therefore, the current experiment was planned to evaluate germplasm's storage potential and to study germination media's influence on short-term stored pollen's germination percentage. This study will standardize pollen storage conditions for each genotype and optimize effective germination media, increasing the effectiveness of future breeding programs.

MATERIAL AND METHODS

Pollen Collection and Pollen Storage

Studies were conducted utilizing fifteen diverse pomegranate genotypes, i.e., Red, Green Khushab, Kanhati, Sandhora Khatta, Tarnab Ghulabi, Tarnab 2, Qibli, BW3, Chakwal, Dasi, Kandhari Red, Kandhari White, Sava, Takht-i-Babri and Sandhora were maintained at pomegranate germplasm block Square No.32, Institute of Horticultural Sciences, University of Agriculture, Faisalabad. To minimize variations due to environmental/agronomic conditions, all the plants were maintained in the same block and subjected to similar agronomic practices. Three uniform, healthy, 6-7 years old pomegranate trees of each genotype were selected for the experiment. For pollen collection, unopened and about to open flower buds at the balloon stage for fifteen pomegranate cultivars were collected, and after removing sepals and petals, flower buds were kept under a 100-Watt lamp for 3-4 hours. The heat from the lamp caused the pollen shedding, and pollen grains were collected in yellow powder form (Figure 1 A,B,C). Collected pollen grains were then thoroughly air dried and kept in sterilized Eppendorf tubes before storage pollen tubes were kept in a desiccator containing non-hydrated calcium carbonate ($CaCO_3$) and were stored in sealed Eppendorf tubes at 4 °C for 30 days (Du *et al*., 2019) (Figure 1 D).

In-vitro Pollen Germination

Pollen viability was assessed using in vitro pollen germination by agar petri method. Germination media containing following three ingredients was utilized:

T1: 12.5% sucrose + 10 ppm boric acid + 0.2% agar
T2: 10% sucrose + 5 ppm boric acid + 0.2% agar
T3: 12.5% sucrose + 0.2% agar

Preparation of Media

Media was prepared by dissolving 10, 12.5, and 12.5 grams of sucrose in 100 ml of distilled water; after toughly dissolving sucrose on the hot plate, 5 and 10 ppm of boric acid was added in two of the treatments, and the pH of the solution was neutralized using pH meter. Then 2 grams of agar was added to each solution for solidification, and the whole media was boiled in the oven. After that, media was transferred into the autoclavable bottle and was autoclaved at 121 °C temperature and 15 psi pressure for 20 minutes along with glass Petri plates. After autoclaving, the media, while in liquid form, was poured into Petri plates under a laminar flow cabinet to avoid contamination. The Petri plates were kept in a laminar flow cabinet until the media was solidified (Maryam et al., 2015).

Culturing and Pollen Counting

Stored pollen grains were taken out of storage and were dusted on germination media, ensuring sterilized conditions. Petri plate edges were closed with paraffin film and kept in the growth room, maintaining 25± 2°C

temperature. After 24 hours, each cultivar's germination ratio was assessed under a microscope. The pollen grains having pollen tube at least two times longer than pollen size was considered to be germinated, and the percentage of germinated pollen grains under three microscopic fields was worked out, which were treated as one replication (Maryam *et al*., 2015) (Figure 1 E, F).

$$\text{Germination Percentage} = \frac{\text{No. of germinated pollens in each field}}{\text{Total No. of pollen grains in each field}} x\ 100$$

Statistical Analysis

The experiment was laid out according to a completely randomized design. Tukey's significant test was used to compare means after ANOVA; P-values of <0.05 were considered significant. For data analysis, Statistics 8.1 was used, and graphs were constructed by MS Excel 2018.

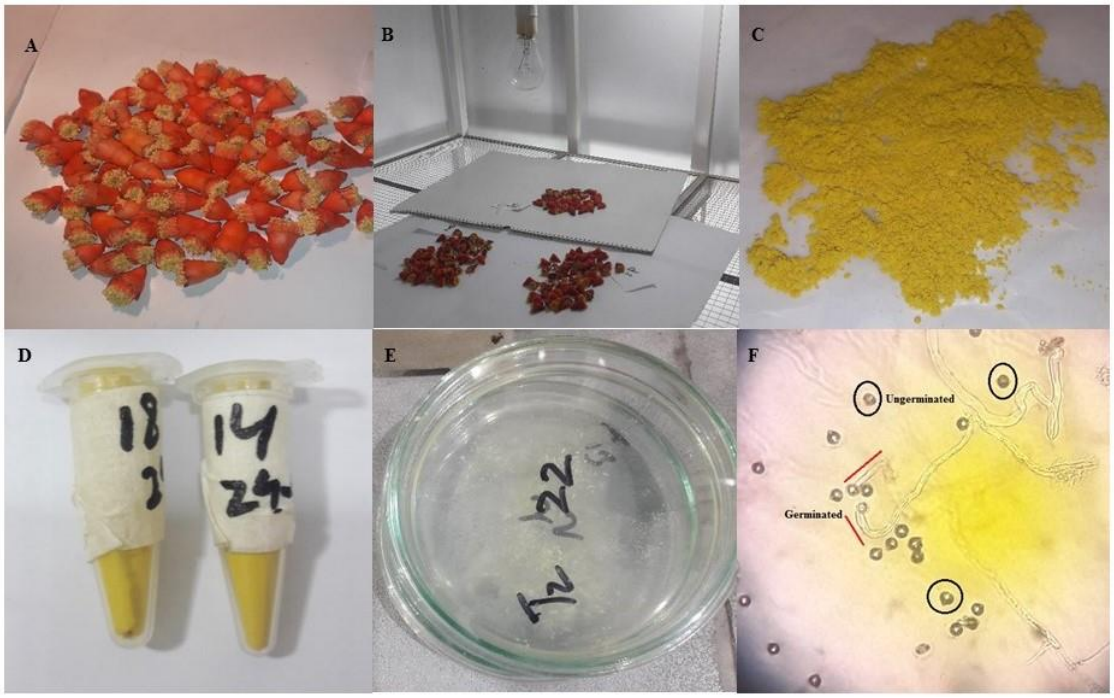

Figure 1. In-vitro pollen germination in pomegranate genotypes. (A) removal of sepals, petals, stigma, and style (B) Anthesis of pollen grains under100-Watt lamp (C) Pollen grains in powder form (D) Storage of pollen grains in Eppendorf tubes at 4 °C for 30 days (E) In-vitro germination using Petri agar method (F) Slide of germinated and ungerminated pollen.

RESULTS AND DISCUSSION

In vitro Pollen Germination in Pomegranate Genotypes

Pomegranate genotypes showed different potential for pollen storage. Genotypes Kandhari White had the highest pollen germination percentage (47.43 %) after 30 days of storage at 4 °C irrespective of the media composition, followed by Kandhari Red (42.84 %) and Dasi (42.31 %), while the minimum germination was found in Sandhora (26.32 %) statistically at par with Takht-i-Babri (27.28 %) (Fig.2). Difference in pollen germination percentage after storage can be due to difference in the genetic makeup of the plant. Variation among genotypes for pollen viability has also been reported for many other crops (Asma, 2008; Dutta *et al*., 2013; Mesnoua *et al*., 2018). The composition and concentrations of starches and proteins in pollen grains can be responsible for pollen viability differences. Plant health, agronomic practices, environmental conditions, collection time, and storage conditions also affect pollen viability and storage potential (Melgarejo *et al*., 2000; Ahmed *et al*., 2017). However, for this experiment, the collection time, storage conditions, agronomic practices, and environmental conditions were uniform for all the

genotypes. Hence the difference observed in viability percentage is due to plant genetic factors.

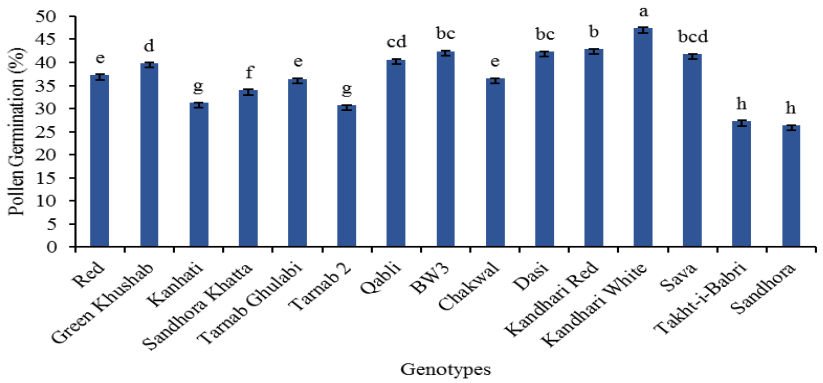

Figure 2. Pollen germination percentage of pomegranate genotypes stored at 4°C for 30 days. Vertical bars indicate ±standard error, and differences in lettering show significance at *n*=5.

Effect of Germination Media on Pollen Germination

The composition of germination media influenced the post-storage germination rate of pomegranate pollens. In germination media highest germination percentage was observed in media containing 12.5 % sucrose + 0.2 % agar + 10 ppm boric acid (42.64 %) followed by media containing 10 % sucrose + 0.2% agar + 5 ppm boric acid (38.72 %) and lowest germination was observed in media containing 12.5 % sucrose + 0.2% agar (30.02 %) (Figure 3). In germination media sucrose provides nutrient for growth of pollen, similar to stigmatic exudate produced in flowers. The concentration of sucrose within the media influences the germination rate. Previously studies have reported that sucrose concentration higher than 20% and lower than 5% results in lower pollen germination rate in pomegranate genotypes (Gadže *et al*., 2011; Üstüntaş *et al*., 2019). Although, sucrose level varies with different plant types. However, for many fruit crops, 10-15% are considered optimum for effective pollen growth (Maryam *et al*., 2015).

Boron has been reported to improve fruit set and pollen tube growth in many fruit crops (Samiee *et al*. 2016; Muengkaew *et al*. 2017). Therefore, to increase the pollen germination rate, media is supplemented with boric acid. The effectiveness of different boric acid concentrations varies with different plant types. In this experiment, boric acid at 10 ppm concentration was found to be more effective for improving pollen germination. Boric acid had a more significant effect on germination rate than sucrose level. These studies are in agreement with (Korkmaz and Guneri, 2019; Melgarejo *et al*., 2000).

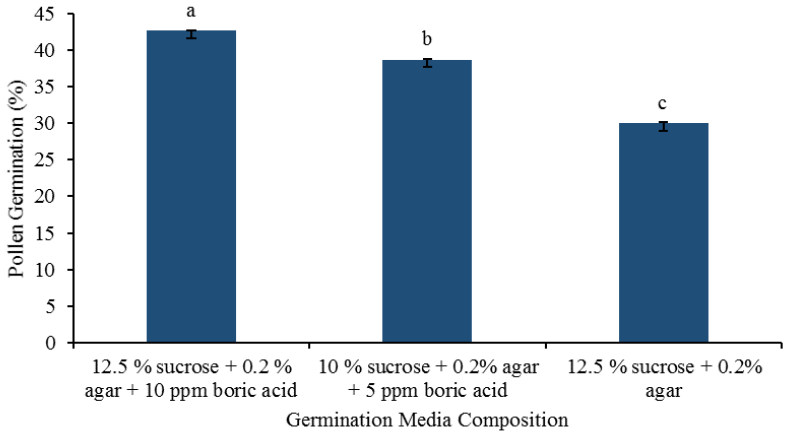

Figure 3. The composition of germination media influences pollen germination percentage. Vertical bars indicate ±standard error, and differences in lettering show significance at *n*=5.

Genotype's Response to different Germination Media

Among genotypes in media containing 12.5 % sucrose + 0.2 % agar + 10 ppm boric acid, the highest pollen germination was observed in Kandhari Red (58.93 %), followed by Kandhari White (58.60 %) and Chakwal (56.50 %) which were not significantly different among each other while the lowest germination was observed in Takht-i-Babri (26.53 %) statistically at par with Sandhora (29.30 %) and Kanhati (30.0 %). In media containing 10 % sucrose + 0.2% agar + 5 ppm boric acid highest germination was observed in BW3 (51.17 %) followed by Sava (46.27 %) and Dasi (46.17 %) while the minimum was observed in Chakwal (27.77 %) statistically at par with Takht-i-Babri (28.60 %). In media containing 12.5 % sucrose + 0.2% agar highest pollen germination was observed in Kandhari White (43.27 %), followed by Red (42.53 %), and minimum germination was observed in Sandhora (14.67 %) (Table 1). Pollen storage is influenced by pollen grains' moisture content, storage temperature differences, and relative humidity. Changes in these conditions affect pollen germination and growth capacity. Pollen drying before storage is useful for pollen preservation as it can prevent the growth and reproduction of microorganisms and minimize many moisture-mediated degradation reactions to enhance shelf-life (Wang *et al*., 2015). Cryo-drying, however, inhibits metabolism and reduces enzyme activity and respiration, which slow the rate at which pollen viability declines. Growth media composition also influences germination (Maryam *et al*., 2015; Du *et al*., 2019).

Table 1. The difference in in vitro pollen germination percentage of pomegranate genotypes in different growing media.

Genotypes	Media types		
	T1	T2	T3
Red	31.6 ± 0.92 n-r	37.50 ± 0.29 kl	42.53 ± 0.59 d-i
Green Khushab	44.4 ± 0.80 cde	41.70 ± 0.40 e-j	33.43 ± 0.30 mno
Kanhati	30.0 ± 0.58 o-s	39.06 ± 0.63 h-k	24.67 ± 0.35 uv
Sandhora Khatta	39.3 ± 0.67 g-k	37.20 ± 1.11 klm	25.33 ± 0.60 tuv
Tarnab Ghulabi	40.16 ± 0.44 f-k	30.33 ± 0.35 o-s	38.83 ± 0.60 i-l
Tarnab 2	37.83 ± 0.44 j-l	31.43 ± 0.40 n-r	22.93 ± 0.52 v
Qabli	43.1 ± 0.20 d-h	45.77 ± 0.43 cd	32.93 ± 0.52 nop
BW3	43.83 ± 0.44 def	51.17 ± 0.72 b	32.33 ± 0.72 n-q
Chakwal	56.50 ± 0.29 a	27.77 ± 0.14 r-u	25.17 ± 0.60 uv
Dasi	48.17 ± 0.60 bc	46.17 ± 0.60 cd	32.60 ± 0.30 n-q
Kandhari Red	58.93 ± 0.23 a	42.43 ± 0.47 d-i	27.17 ± 0.60 stu
Kandhari White	58.60 ± 0.21 a	40.43 ± 0.74 e-k	43.27 ± 0.67 d-g
Sava	51.30 ± 0.62 b	46.27 ± 0.72 cd	27.73 ± 0.43 r-u
Takht-i-Babri	26.53 ± 0.67 s-v	28.60 ± 2.65 q-u	26.70 ± 1.71 s-v
Sandhora	29.30 ± 0.46 p-t	35 ± 0.23 lmn	14.67 ± 0.47 w

Mean+SE, difference in lettering show significance at *n*=5.
*T1: 12.5 % sucrose + 0.2 % agar + 10 ppm boric acid
*T2: 10 % sucrose + 0.2% agar + 5 ppm boric acid
*T3: 12.5 % sucrose + 0.2% agar

CONCLUSION

Pomegranate pollens can be successfully stored at 4 °C for 30 days. The post-storage germination rate varies among genotypes and germination media composition. The germination percentage ranged from 47.43%-26.33% among genotypes. Germination media consisting of 12.5 % sucrose + 0.2 % agar + 10 ppm boric acid was found to be most effective for higher pollen germination.

AUTHOR'S CONTRIBUTIONS

Ikram S: Experiment design, Fieldwork, Data analysis,

and First draft; Ikram S: Fieldwork and First draft; Rehman SU: Fieldwork, First draft; Hussain M: Fieldwork, First draft; Qureshi MA: Data analysis, Manuscript revision, and final draft; Shafqat W: Experiment design, Data analysis, Manuscript revision; Din SU: Data analysis, Manuscript revision; Zafar MS: Helped in Data Analysis, Raza S: Helped in Data Analysis, Bukhari SIU: Helped in Data Analysis.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Ahmed, S., H.S. Rattanpal, E. Ahmad and G. Singh. 2017. Influence of Storage Duration and Storage Temperature on In-Vitro Pollen Germination of Citrus Species. international journal of current microbiology and applied sciences. 6:892–902.

Anonymous. 2020. 2020-2025 Global Pomegranate Concentrate Market Report - Production and Consumption Professional Analysis (Impact of COVID-19). https://www.industryresearch.co/enquiry/request-sample/17457870.

Asma, B.M. 2008. Determination of pollen viability, germination ratios and morphology of eight apricot genotypes. African Journal of Biotechnology. 7(23):4269-4273.

Astarini, I.A. 2019. Pollen Storage and Viability of Twelve Citrus Genotypes in Bali, Indonesia. SCR Biology, 2:55-62.

Babu, K.D. 2010. Floral Biology of Pomegranate (*Punica granatum* L.). Globle Science. 4:45–50.

Chandra, R., D.K. Babu, V.T. Jadhav and J.A. Teixeira da Silva. 2010. Origin, history and domestication of pomegranate. Fruit, Veg. Cereal Science. Biotechnology. 4:1–6.

Du, G., J. Xu, C. Gao, J. Lu, Q. Li, J. Du, M. Lv and X. Sun. 2019. Effect of low storage temperature on pollen viability of fifteen herbaceous peonies. Biotechnology. Reports 21:e00309.

Dutta, S.K., M. Srivastav, R. Chaudhary, K. Lal, P. Patil, S.K. Singh and A.K. Singh. 2013. Low temperature storage of mango (*Mangifera indica* L.) pollen. Science Horticulture (Amsterdam), 161:193–197.

Gadže, J., M. Radunić, I.V. Petric and S. Ercisli. 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.

Graham, S.A., J. Hall, K. Sytsma and S.H. Shi. 2005. Phylogenetic analysis of the Lythraceae based on four gene regions and morphology. International journal of plant sciences, 166:995–1017.

Holland, D., K. Hatib and I. Bar-ya. 2009. Pomegranate: Botany, horticulture, breeding. Horticultural Reviews, 35(2):127-192.

Ikram, S., W. Shafqat, M.A. Qureshi, S.U. Din, S.U. Rehman, A. Mehmood, Y. Sajjad and M. Nafees. 2020. Causes and control of fruit cracking in pomegranate: A review. Journal of global innovations in agricultural social sciences, 8(4):183-190.

Korkmaz, N. and M. Guneri. 2019. Effect of different boron levels on pollen germination of hicaz nar pomegranate (*Punica granatum* l.) Cultivar. International Journal of Agriculture and Biology, Forestry Life Sci. 3:151–156.

Li, Y.-M. and L.-B. Chen. 1998. Vigor change of several grasses pollen stored in different temperature and humidity conditions. plant physiology communications, 34:35–37.

Maryam, M.J. Jaskani, B. Fatima, M.S. Haider, S.A. Naqvi, M. Nafees, R. Ahmad and I.A. Khan. 2015. Evaluation of pollen viability in date palm cultivars under different storage temperatures. Pakistan Journal of Botany, 47:377–381.

Melgarejo, P., P. Legua, M. Martinez and J.J. Martinez. 2000. Contribution to a better knowledge of the quality of pomegranate pollen (*Punica granatum* L.). Options Méditerranéennes. Série A, Séminaires Méditerranéens 115–121.

Mesnoua, M., M. Roumani and A. Salem. 2018. The effect of pollen storage temperatures on pollen viability, fruit set and fruit quality of six date palm cultivars. Science Horticulture (Amsterdam), 236:279–283.

Mir, M.M., I. Umar, S.A. Mir, M.U. Rehma, G.H. Rather and S.A. Banday. 2012. Quality evaluation of pomegranate crop -A review. International Journal of Agriculture and Biology, 14:658–667.

Muengkaew, R., P. Chaiprasart and P. Wongsawad. 2017. Calcium-Boron Addition Promotes Pollen Germination and Fruit Set of Mango. International Journal of Fruit Science, 17:147–158.

Samiee R. A., A. Imani and M. Salmani. 2016. The

Interaction Effects of Boron and Plant Growth Regulators on Pollen Germination of Almond. Int. J. Nuts and Related Sci. 7:445-50.

Üstüntaş, T., Z. Dalkılıç and G. Günver Dalkılıç. 2019. Effect of stigma exudate on pollen germination in pomegranate. Acta Horticulture, 1254:109–114.

Wang, L., J. Wu, J. Chen, D. Fu, C. Zhang, C. Cai and L. Ou. 2015. A simple pollen collection, dehydration, and long-term storage method for litchi (*Litchi chinensis* Sonn.). Science Horticulture (Amsterdam), 188:78–83.

Yuan, Z., Y. Fang, T. Zhang, Z. Fei, F. Han, C. Liu, M. Liu, W. Xiao, W. Zhang, S. Wu, M. Zhang, Y. Ju, H. Xu, H. Dai, Y. Liu, Y. Chen, L. Wang, J. Zhou, D. Guan, M. Yan, Y. Xia, X. Huang, D. Liu, H. Wei and H. Zheng. 2018. The pomegranate (*Punica granatum* L.) genome provides insights into fruit quality and ovule developmental biology. Plant Biotechnology Journal, 16:1363–1374.