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Research Article

Storage and *In-vitro* Germination of Some Olive Pollens

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Abstract: In this study, the effect of storage at different times and temperatures on *in vitro* olive pollen germination was investigated. Pollens of Gemlik and Domat cultivars and GE119 and GK138 genotypes were stored at +4 °C, -20 °C, and -80 °C for 7, 35, 200, and 365 days, respectively. *In vitro* germination status of pollen was determined by the petri agar method, by choosing the most suitable nutrient medium for each olive. The most suitable germination media for Gemlik, Domat, GE119, and GK138 pollens were 50 ml water + 15% sucrose + 0.7% agar + 75 ppm boric acid, 50 ml water + 15% sucrose + 0.7% agar, 50 ml water + 25% sucrose + 0.5% agar, 50 ml water respectively. The interaction effect between storage time, temperatures, and cultivar on pollen germination and diameter was determined. At the end of the storage period, the highest pollen germination and diameter were observed in the Gemlik cultivar. Additionally, -80 °C temperature for Gemlik and Domat cultivars and -20 °C temperature for GE119 and GK138 genotypes were suitable for 35 days of storage. All olive pollens in the current study had germination rates below 9% in the following storage periods. The results show that storing olive pollens at sub-zero temperatures will reduce the need for daily fresh pollen collection required for important scientific studies such as breeding and artificial pollination.

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1. Introduction

The olive tree (*Olea europaea* L.) is known as the most iconic tree of the world, originating about six thousand years ago, emerging in connection with the emergence of many civilizations in Upper Mesopotamia and Southern Asia Minor (Zohary et al., 2012; Besnard et al., 2018). Semites carried out the first olive cultivation, and this situation spread to the Aegean Sea Islands, Anatolia, Egypt, Greece, Italy, Morocco, Spain, and Tunisia (Özkaya et al., 2008). It has great socio-economic importance in the Mediterranean basin (Topaklı and Hepaksoy, 2019). Olive and olive oil consumption is increasing due to health components and many high-quality olive cultivars are grown in olive growing regions (Rallo et al., 2018; Shavakhi et al., 2021). However, as in every fruit species, some problems arise in olive cultivation. Since parthenocarpic fruits have no commercial value in olives, pollination is needed for proper yield (Koubouris et al., 2009). In addition, olives show absolute periodicity and yield decreases

over the years. Similar issues are seen in olive orchards that are not established with the appropriate pollinator due to low self-productivity (Alagna et al., 2019; Dölek Gencer and Özkaya, 2020). Furthermore, scientists working with olives emphasize the importance of cross-pollination to increase fruit set, especially in partially self-fertile and self-sterile cultivars (Sanchez-Estrada and Cuevas, 2018; Sanchez-Estrada and Cuevas, 2019). Cross-pollination is therefore a mandatory practice for completely self-infertile cultivars. For all these reasons, biological or mechanical artificial pollination with pre-collected and stored pollen together with the selection of suitable pollinators for high fruit set is considered an important cultural process in olive production (Sanchez-Estrada and Cuevas, 2020).

Pollen is always needed to pollinate olives due to the lack of sample flowering caused by structural in the cultivars and the fact that the flowering periods of the cultivars with very different flowering dates do not coincide. For this, pollen retention is of particular importance. Only at low temperatures can high pollen viability and germination ratings be protected for an extended period of time. Hechmi et al. (2015) found that the pollens of Koroneik, Frantoio, Manzanille, and Nabali olive cultivars all have a great tolerance to low temperatures (-20 and 10 °C). These pollens showed decreasing germination during storage periods and this situation was clearly evident even after 1 month of storage. And also, pollen deaths were observed after 1 month in pollens stored at 25 °C, while deaths occurred after 180 days in pollen stored at -20 and +10 °C.

Pollen storage and germination are the subjects of studies for plant breeding, conservation of gene resources, and examining the physiological conditions of different species. Artificial pollination is a necessity in fruit growing, especially in hybridization breeding and the selection of suitable pollinators. For this reason, pollen storage has a special importance in terms of providing the pollen needed at different times. Besides, pollen storage is considered necessary for the preservation of olive genetic resources and their use in olive improvement programs. In the studies conducted with different fruit species, it is stated that pollen can be stored for 1-3 months at 4 °C, but temperatures below 0 °C are more suitable for longer storage (El Kadri and Ben Mimoun, 2020; Özcan, 2020; Özcan and Bükücü, 2020). Moreover, pollen germination after cold storage is reported to differ even among cultivars of the same species (Kuroki et al., 2017; Quinet and Jacquemart, 2020), while germination levels can vary according to factors such as the nutrient content of the germination medium, humidity, temperature and pH (Mertoğlu et al., 2018; Kılıç et al., 2020; Güçlü et al., 2021; Fagundes et al., 2021; Đorđević et al., 2022).

It was intended to find the best germination medium for pollen belonging to different olive cultivars and genotypes under *in vitro* conditions and to determine the germination levels with pollen swelling changes after storing these pollens at different temperatures.

2. Material and Methods

2.1. Material

Pollen of Gemlik and Domat olive cultivars and GE119 (Gemlik × Edincik Su) and GK138 (Gemlik × Karamürsel Su) olive genotypes were grown in Atatürk Horticultural Central Research Institute, were used as materials.

2.2. Method

Laboratory studies were carried out together with Ankara University, Faculty of Agriculture, Department of Horticulture, and Bülent Ecevit University in 2015-2016. Pollen was collected early in the morning when the flowers reached the balloon stage and brought to the laboratory under appropriate conditions. After the petals were removed, the filaments were separated with forceps and kept at 20 °C for 24 hours. Due to its practicality, the petri dish agar method was used to determine in-vitro pollen germination rates (Koyuncu, 2006). In order to find the most suitable nutrient medium for each cultivar and genotype, 23 different combinations of water, sucrose, boric acid, and calcium chloride were studied (Table 1). The sown pollens were taken into ovens at a temperature of 24 °C determined as a result of the preliminary trials, and *in vitro* germination rates were checked after 3 hours. Pollen diameters were observed after 24 hours under a Leica light microscope at 40 magnification using an ocular micrometer. Pollens were stored at +4 °C, -20 °C, and -80 °C for 7, 35, 200, and 365 days.

Table 1. Nutrient media combinations used in *in vitro* germination

Nutrient No	Nurtient Name	Nurtient Concentrations
1	**Z1**	50 ml water
2	**Z2**	50 ml water + %10 sucrose + %0.7 agar
3	**Z3**	50 ml water + %10 sucrose + %0.5 agar
4	**Z4**	50 ml water + %10 sucrose + %0.3 agar
5	**Z5**	50 ml water + %10 sucrose + %0.1 agar
6	**Z6**	50 ml water + %15 sucrose + %0.7 agar
7	**Z6A**	50 ml water + %15 sucrose + %0.7 agar + 75 ppm boric acid
8	**Z6B**	50 ml water + %15 sucrose + %0.7 agar + 100 ppm boric acid
9	**Z6C**	50 ml water + %15 sucrose + %0.7 agar + 125 ppm boric acid
10	**Z6D**	50 ml water + %15 sucrose + %0.7 agar + %0.01 calcium chloride
11	**Z6E**	50 ml water + %15 sucrose + %0.7 agar + %0.05 calcium chloride
12	**Z6F**	50 ml water + %15 sucrose + %0.7 agar + %0.1 calcium chloride
13	**Z7**	50 ml water + %15 sucrose + %0.5 agar
14	**Z8**	50 ml water + %15 sucrose + %0.3 agar
15	**Z9**	50 ml water + %15 sucrose + %0.1 agar
16	**Z10**	50 ml water + %20 sucrose + %0.7 agar
17	**Z11**	50 ml water + %20 sucrose + %0.5 agar
18	**Z12**	50 ml water + %20 sucrose + %0.3 agar
19	**Z13**	50 ml water + %20 sucrose + %0.1 agar
20	**Z14**	50 ml water + %25 sucrose + %0.7 agar
21	**Z15**	50 ml water + %25 sucrose + %0.5 agar
22	**Z16**	50 ml water + %25 sucrose + %0.3 agar
23	**Z17**	50 ml water + %25 sucrose + %0.1 agar

2.3. Statistical analysis

The study was conducted according to the randomized plot design with 3 replications and 3 petri dishes in each replication. Each petri dish was divided into 3 zones and 250 pollens were counted in these zones. The data obtained in the study were analyzed with separate analyses. Firstly, a two-way ANOVA was applied according to the model: $Yijk = \mu + OCi + NCj + (OC \times NC)ij + eijk$, where:

Yijk is the dependent variable,
μ is the overall mean,
OCi is the olive cultivar or genotype (i = Gemlik, Domat, GE119, or GK138)
NCj is the nutrient medium (j = Z1, Z2,….., Z16, or Z17)
(OC × NC)ij is the interaction between the olive cultivar or genotype and the nutrient medium and eijk is the residual error term.

After that, the nutrient medium was selected for each cultivar or genotype and pollens to determine the best storage temperature and duration and the study was a 4 × 3 × 4 factorial design with 4 olive cultivars or genotypes, 3 storage temperatures, and 4 storage durations. Data were analyzed via a GLM procedure in the Minitab Version 17 package program (Minitab, Inc., State College, PA). Significant differences that emerged at the end of the variance analysis were compared with Tukey's test ($P \leq 0.05$) with MSTAT-C statistical software. The results were expressed as mean values with standard error means (SEM). Cultivar and germination medium factors in determining the best germination medium; cultivar or genotype, storage time, and storage temperature factors and their interactions in the determination of pollen germination rate and diameter during storage were taken into account as variables.

3. Results and Discussion

Various chemicals are needed for optimal pollen germination and tube growth. It is important to determine the most suitable germination environment in different species and even between different genotypes of the same species, usually by modifying the concentrations of these chemicals. In our study, pollen planted in different nutrient media swelled in the form of beads, and germination was observed with pollen tubes within 1 hour in Gemlik pollen as seen in pear pollen (Vasilakakis and Porlingis, 1985) and within 3 hours in other cultivars and genotypes (Figure 1 and 2).

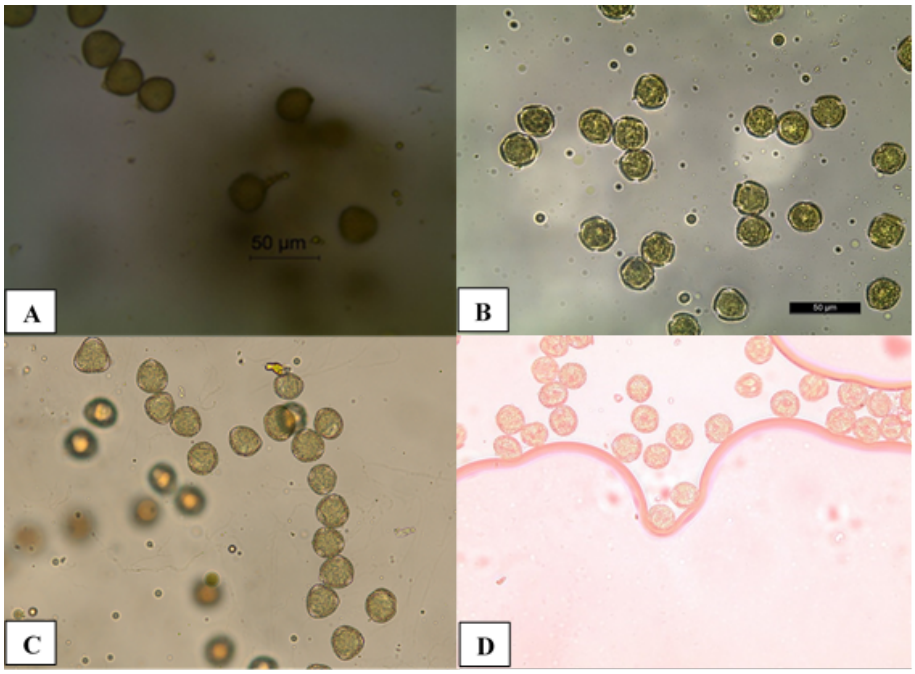

Figure 1. Swelling of pollens Gemlik (A), Domat (B), GE 119 (C), GK 138 (D).

The interaction of cultivar and medium appears to be important in the germination of olive pollen on the 10th day in different nutrient media (P = 0.001). Z6A (50 ml water + 15% sucrose + 0.7% agar + 75 ppm boric acid) for Gemlik, Z6 (50 ml water + 15% sucrose + 0.7% agar) for Domat, Z15 (50 ml water + 25% sucrose + 0.5% agar) for GE119 and Z1 (50 ml water) for GK138 were determined to be the best combinations for pollen germination (Table 2). In these nutrient media, a germination rate of 45% in the pollen of the Gemlik cultivar, 44% in the Domat cultivar, 42% in the GE119 genotype, and 27% in the GK138 genotype was observed. In addition, the germination levels of olive pollen differed for each medium. In this context, it is known that the effect of different nutrient media on pollen germination differs according to plant species and cultivars (Altunbaş and Engin, 2016).

The interaction of cultivar × storage time × storage temperature is important for *in vitro* germination of olive pollen stored at different temperatures and storage times (P = 0.000) (Table 3).

When the differences between the cultivars were examined in terms of pollen germination rates for each storage period and storage temperature, it was seen that the pollen of the Gemlik cultivar germinated more than other pollens at all storage temperatures during the 7th day of storage, and the GK138 genotype showed similar germination at only +4 °C for the same period. While the Gemlik cultivar and GE 119 genotype showed a high germination rate at both +4 °C and -20 °C temperatures on the 35th day, only the Gemlik cultivar achieved a high germination rate at -80 °C in the same period. All olive pollens had similar germination after 200 days of storage at -20 °C, but Gemlik showed the highest germination rate among the cultivars at -80 °C for the same period. Decay was observed in all olive pollens stored at +4 °C on the 365th day of storage. No germination was seen in GE 119 and GK138 genotypes stored at -20 °C for 365 days and in GE 119 genotypes stored at -80 °C for the same time. Gemlik pollens had higher germination rates than other olive pollens, and GK138 pollens had lower germination rates. Similarly, it is stated in many studies that different cultivars and genotypes of the same species have different pollen germination rates (Ferri et al., 2008; Bhat et al., 2012; Mesnoua et

al., 2018; Aldahadha et al., 2019). Considering cultivars and genotypes, it is thought that there is a strong genetic influence on pollen germination.

Table 2. *In vitro* germination percentages of olive pollen on day 0th in different nutrient media

Nutrient Media	Gemlik	Domat	GE119	GK138
Z1	17 ± 2.31 C, cdef*	32 ± 2.31 A, b*	20 ± 2.31 BC, bcde*	27 ± 2.31 AB, a*
Z2	3 ± 1.73 C, gh	25 ± 2.31 A, bcd	14 ± 2.31 B, defg	5 ± 2.31 C, cd
Z3	8 ± 2.31 A, fgh	11 ± 2.31 A, efgh	6 ± 2.31 A, gh	12 ± 2.31 A, bc
Z4	1 ± 0.57 C, h	17 ± 2.31 B, cdef	26 ± 2.31 A, bc	20 ± 2.31 AB, ab
Z5	9 ± 2.31 B, fgh	16 ± 2.31 AB, cdefg	20 ± 2.31 A, bcde	9 ± 2.31 B, bcd
Z6	17 ± 2.31 B, cdef	44 ± 2.31 A, a	18 ± 2.31 B, bcdef	12 ± 2.31 B, bc
Z6A	45 ± 2.31 A, a	6 ± 2.31 C, fgh	20 ± 2.31 B, bcde	0 ± 0.00 C, d
Z6B	12 ± 2.31 A, efgh	0 ± 0.00 B, h	9 ± 2.31 A, efgh	4 ± 2.31 AB, cd
Z6C	26 ± 2.31 A, bc	17 ± 2.31 B, cdef	8 ± 2.31 C, fgh	8 ± 2.31 C, cd
Z6D	17 ± 2.31 A, cdef	13 ± 2.31 A, efg	10 ± 2.31 A, efgh	9 ± 2.31 A, bcd
Z6E	17 ± 2.31 AB, cdef	19 ± 2.31 A, cde	5 ± 2.31 C, gh	10 ± 2.31 BC, bcd
Z6F	24 ± 2.31 A, bcd	14 ± 2.31 B, defg	7 ± 2.31 B, egh	12 ± 2.31 B, bc
Z7	15 ± 2.31 B, cdef	14 ± 2.31 B, defg	28 ± 2.31 A, b	0 ± 0.00 C, d
Z8	32 ± 2.31 A, b	18 ± 2.31 B, cde	16 ± 2.31 B, cdefg	20 ± 2.31 B, ab
Z9	12 ± 2.31 B, efgh	26 ± 2.31 A, bc	10 ± 2.31 B, efgh	0 ± 0.00 C, d
Z10	9 ± 2.31 A, fgh	5 ± 2.31 A, gh	2 ± 1.15 A, h	9 ± 2.31 A, bcd
Z11	22 ± 2.31 A, bcde	10 ± 2.31 B, efgh	16 ± 2.31 AB, cdefg	9 ± 2.31 B, bcd
Z12	25 ± 2.31 B, bc	36 ± 2.31 A, ab	14 ± 2.31 C, defg	8 ± 2.31 C, cd
Z13	9 ± 2.31 C, fgh	33 ± 2.31 A, ab	23 ± 2.31 B, bcd	12 ± 2.31 C, bc
Z14	10 ± 2.31 B, fgh	18 ± 2.31 B, cde	28 ± 2.31 A, b	0 ± 2.31 C, d
Z15	13 ± 2.31 B, defg	15 ± 2.31 B, cdefg	42 ± 2.31 A, a	15 ± 2.31 B, bc
Z16	15 ± 2.31 A, cdef	12 ± 2.31 A, efg	9 ± 2.31 A, efgh	0 ± 0.00 B, d
Z17	24 ± 2.31 A, bcd	0 ± 0.00 C, h	9 ± 2.31 B, efgh	0 ± 0.00 C, d

*: mean ± standard error. The capital letters in the first row represent the differences between cultivars in each nutrient medium, and the lower letters in the second row represent the differences between the nutrient media for each cultivar at $P \leq 0.05$.

When the differences between temperatures for each cultivar and storage period are examined, pollen germination rates at all temperatures on the 7th day for the Gemlik cultivar were in the same statistical group, while the positive effects of -20 °C and -80 °C temperatures in other storage processes were observed. It was determined that only pollens stored at -80 °C on the 35th day showed higher germination in the Domat cultivar, and all temperatures had similar effects on the other days. Seven days of storage at +4 °C and 35 days of storage at -20 °C were found to be effective for the GE 119 and the GK138 genotypes, while the other days were ineffective. Similar to our study, Özcan (2020) stated that storing pollens of different cherry cultivars at temperatures below -20 °C had a positive effect on germination compared to higher temperatures. In general, germination rates of pollens stored at temperatures above zero are lower than those stored below zero (Pham et al., 2015). However, it is specified that +4 °C temperature is suitable for the preservation of some pollens under suitable conditions (Martínez-Gómez et al., 2000). This situation is thought to be caused by the differences in genetic characteristics of species and cultivars. In our study, the ineffectiveness of -20 °C and -80 °C temperatures in the GK138 genotype can be ascribed to the unfavorable effects of cell injuries that occur when some pollens freeze and thaw at sub-zero temperatures (Luza and Polito, 1988).

When the differences between storage times for each cultivar and storage temperature were examined, decreases in in-vitro pollen germination rates were determined after 200 days at the same temperatures for all cultivars. Especially in the 200th and 365th days analyses, low germination rates below 9% were observed in all pollens. Similar to our study, Mortazavi et al. (2010) reported that palm pollens did not germinate after 200 days of cold storage, but Martinez-Gomez et al. (2002) stated that different almond cultivars' pollens had germination rates of over 40% in 1-year storage period at subzero temperatures. In our study, the highest germination rates were observed in Gemlik (59%) and Domat (24%) cultivars stored at -80 °C for 35 days, in the GE 119 (40%) genotype stored at -20 °C for 35 days, and in the GK 138 genotype stored at +4 °C for 7 days. Especially after the 35th day of storage, pollen germination rates, which decrease with the progress of storage, are compatible with other studies (Bolat

and Güleryüz, 1994). In a study conducted with the Manzanillo olive cultivar, the germination of pollens stored at -20 °C decreased by 40% after 365 days (Pinney and Polito, 1990). Besides that, it has been reported that the pollens of the Arbequina olive cultivar can be stored for 60 days at -10 °C (Zambon et al., 2018).

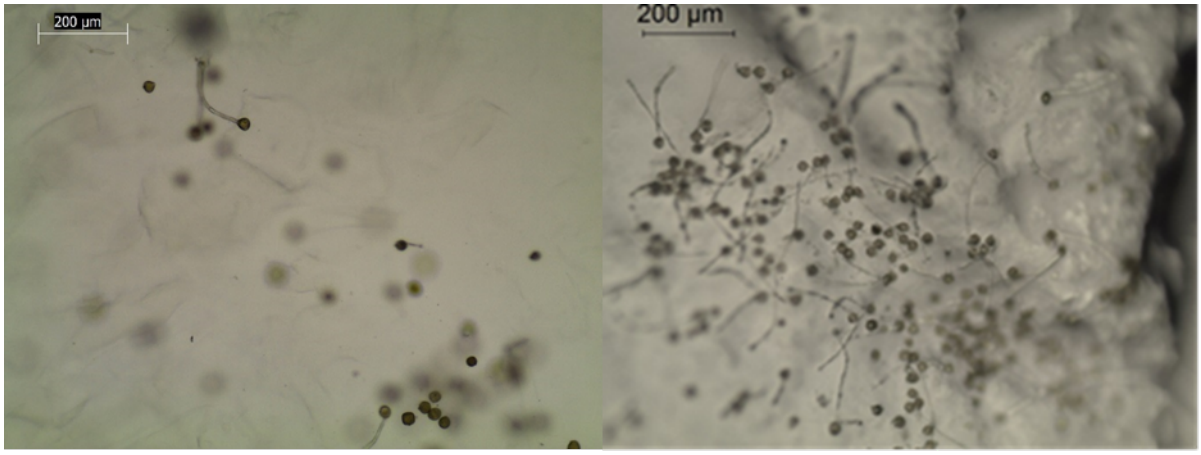

Figure 2. Pollen tube formation and germination in Gemlik cultivar.

The interaction of cultivar × storage time × storage temperature was important for the effect of the storage of olive pollens at different temperatures and storage times on pollen diameters ($P <= 0.001$) (Table 3). During the study, pollen diameters were determined between 18.14 - 25.80 µm. These values are seen as close values for Italian (17-20 µm) (Isocrono and Vallania, 2009), Greek (14-22 µm) (Koubouris et al., 2012), and Iranian olive (15-29 µm) (Javady and Arzani, 2001) cultivars.

When the differences between cultivars were examined in terms of pollen diameters for each storage period and storage temperature, it was observed that the Gemlik cultivar had higher pollen diameters in 7 days of storage at +4 °C and -20 °C, and the GK 138 pollen diameters in 7 days of storage at -80 °C. While GK 138 genotype had lower pollen diameters at +4 °C and -20 °C temperatures in 35th day samples, low pollen diameter values were determined in the GK 138 and Domat pollen at -80 °C in the same storage period. The highest pollen diameter was observed in the pollen of the GE 119 genotype in the samples stored on the 200th day at all temperatures. Among the pollens stored at -20 °C and -80 °C for 365 days, GK 138 had the lowest pollen diameter.

When the differences between temperatures for each cultivar and storage period were examined, it was discovered that +4 °C temperature for Gemlik and GE 119 pollens, as well as +4 °C and -80 °C temperatures for Domat and GK 138 pollens, were effective on pollen diameter values on the 7th day of storage. While Gemlik, GE 119, and GK 138 pollen diameters were higher after 35 days of storage at -80 °C, higher pollen diameters were determined for Domat at +4 °C during the same storage period. During storage periods of 200 and 365 days, temperatures of -20 °C and -80 °C for Gemlik and Domat cultivars, -80 °C for the GE 119 genotype, and +4 °C and -20 °C for the GK 138 genotype were effective in terms of pollen diameter.

When the differences between the storage times for each cultivar and storage temperature were analyzed, it was observed that the diameter of the pollen decreased with the progression of the storage period for all pollens stored at +4 °C. The changes in the pollen diameters after the 35th day at -20 °C temperature in the Gemlik cultivar were statistically insignificant, and the values were in the same statistical group in all storage processes at -80 °C. The highest pollen diameter in the Domat cultivar was determined on the 365th day of storage at -20 °C and on the 7th day of storage at -80 °C. For the GE 119 genotype, the diameters of the pollen stored at -20 °C and -80 °C were found to be high on the 200th and 365th days. In the GK 138 genotype, high pollen diameters were determined on the 200th day of storage at -20 °C and on the 7th day at -80 °C.

Table 3. *In vitro* germination rate and diameters of olive pollens during storage at different temperatures

Cultivar × Storage Time × Storage Temperature	Pollen Germination Rate (%)	Pollen Diameter (µm)
Gemlik × 7. days × +4 °C	32 ± 2.31 AB,a,A*	25.80 ± 0.00 A,a,A*
Domat × 7. days × +4 °C	11 ± 2.31 C,b,A	23.62 ± 0.04 C,a,A
GE119 × 7. days × +4 °C	27 ± 2.31 B,a,A	25.42 ± 0.25 AB,a,A
GK138 × 7. days × +4 °C	37 ± 2.31 A,a,A	25.00 ± 0.00 B,a,A
Gemlik × 7. days × -20 °C	35 ± 2.31 A,a,A	22.72 ± 0.10 A,b,A
Domat × 7. days × -20 °C	18 ± 2.31 B,a,A	20.47 ± 0.47 B,b,B
GE119 × 7. days × -20 °C	10 ± 2.31 C,b,B	19.45 ± 0.02 C,b,C
GK138 × 7. days × -20 °C	16 ± 2.31 BC,b,A	18.89 ± 0.21 C,b,C
Gemlik × 7. days × -80 °C	38 ± 2.31 A,a,B	21.53 ± 0.50 C,c,A
Domat × 7. days × -80 °C	8 ± 2.31 C,b,B	23.92 ± 0.24 B,a,A
GE119 × 7. days × -80 °C	16 ± 2.31 B,b,A	18.75 ± 0.00 D,c,C
GK138 × 7. days × -80 °C	11 ± 2.31 BC,b,A	25.00 ± 0.00 A,a,A
Gemlik × 35. days × +4 °C	33 ± 2.31 A,c,A	21.42 ± 0.13 A,a,B
Domat × 35. days × +4 °C	16 ± 2.31 B,b,A	21.58 ± 0.19 A,a,B
GE119 × 35. days × +4 °C	27 ± 2.31 A,b,A	21.14 ± 0.13 A,a,C
GK138 × 35. days × +4 °C	0 ± 0.00 C,b,B	0.00 ± 0.00 B,b,B
Gemlik × 35. days × -20 °C	41 ± 2.31 A,b,A	21.50 ± 0.25 A,a,B
Domat × 35. days × -20 °C	14 ± 2.31 B,b,A	20.93 ± 0.09 A,b,AB
GE119 × 35. days × -20 °C	40 ± 2.31 A,a,A	20.91 ± 0.06 A,a,B
GK138 × 35. days × -20 °C	10 ± 2.31 B,a,A	18.84 ± 0.13 B,a,C
Gemlik × 35. days × -80 °C	59 ± 2.31 A,a,A	21.08 ± 0.12 A,a,A
Domat × 35. days × -80 °C	24 ± 2.31 B,a,A	17.64 ± 0.00 B,c,C
GE119 × 35. days × -80 °C	16 ± 2.31 C,c,A	20.57 ± 0.25 A,a,B
GK138 × 35. days × -80 °C	6 ± 2.31 D,ab,AB	17.64 ± 0.00 B,a,C
Gemlik × 200. days × +4 °C	0 ± 0.00 A,b,B	21.39 ± 0.20 B,a,B
Domat × 200. days × +4 °C	0 ± 0.00 A,a,B	20.15 ± 0.16 C,b,C
GE119 × 200. days × +4 °C	0 ± 0.00 A,a,B	22.21 ± 0.18 A,b,B
GK138 × 200. days × +4 °C	0 ± 0.00 A,a,B	0.00 ± 0.00 D,c,B
Gemlik × 200. days × -20 °C	5 ± 2.31 A,ab,B	21.18 ± 0.31 B,a,B
Domat × 200. days × -20 °C	4 ± 2.31 A,a,B	21.07 ± 0.14 B,a,AB
GE119 × 200. days × -20 °C	5 ± 2.31 A,a,BC	22.03 ± 0.18 A,b,A
GK138 × 200. days × -20 °C	3 ± 1.73 A,a,B	21.25 ± 0.03 B,a,A
Gemlik × 200. days × -80 °C	9 ± 2.31 A,a,C	21.08 ± 0.10 B,a,A
Domat × 200. days × -80 °C	1 ± 0.57 B,a,C	20.78 ± 0.15 B,a,B
GE119 × 200. days × -80 °C	3 ± 1.73 AB,a,B	23.83 ± 0.40 A,a,A
GK138 × 200. days × -80 °C	1 ± 0.57 B,a,B	19.32 ± 0.12 C,b,B
Gemlik × 365. days × +4 °C	0 ± 0.00 A,b,B	0.00 ± 0.00 A,b,C
Domat × 365. days × +4 °C	0 ± 0.00 A,a,B	0.00 ± 0.00 A,b,D
GE119 × 365. days × +4 °C	0 ± 0.00 A,a,B	0.00 ± 0.00 A,c,D
GK138 × 365. days × +4 °C	0 ± 0.00 A,a,B	0.00 ± 0.00 A,c,B
Gemlik × 365. days × -20 °C	8 ± 2.31 A,a,B	21.71 ± 0.07 A,a,B
Domat × 365. days × -20 °C	2 ± 1.15 AB,a,B	21.41 ± 0.25 A,a,A
GE119 × 365. days × -20 °C	0 ± 0.00 B,a,C	21.78 ± 0.07 A,b,A
GK138 × 365. days × -20 °C	0 ± 0.00 B,a,B	19.88 ± 0.24 B,a,B
Gemlik × 365. days × -80 °C	5 ± 2.31 A,ab,C	21.52 ± 0.05 B,a,A
Domat × 365. days × -80 °C	3 ± 1.73 A,a,BC	21.39 ± 0.30 B,a,B
GE119 × 365. days × -80 °C	0 ± 0.00 A,a,B	24.35 ± 0.07 A,a,A
GK138 × 365. days × -80 °C	3 ± 1.73 A,a,B	18.14 ± 0.07 C,b,C
Significant Effects		
Cultivar (C)	< 0.001	< 0.001
Temperature (T)	< 0.001	< 0.001
Storage Time (ST)	< 0.001	< 0.001
C × T	< 0.001	< 0.001
C × ST	< 0.001	< 0.001
T × ST	< 0.001	< 0.001
C × T × ST	< 0.001	< 0.001

*: mean ± standard error. Capital letters in the first row show the differences between cultivars for each storage period and storage temperature, lower letters in the second row show the differences between storage temperatures for each storage period and cultivar, and capital letters in the third row show the differences between storage times for each cultivar and storage temperature according to the Tukey test. ≤ 0.05 refers to the error level.

In the current study, the cultivar, storage temperature, and duration were effective in terms of pollen diameters. However, it is stated that these variables were ineffective on Olivo della Strega olive pollen diameters (Petruccelli et al., 2021).

Conclusion

It is thought that 35 days of storage for Gemlik, Domat, and GE119 pollens, and 7 days of storage for GK138 pollens, were considered appropriate. All cultivars and genotypes had very low germination ability after 200 and 365 days of storage. In terms of storage temperature, the pollen of Gemlik and Domat cultivars had higher germination ability with storage at -80 °C, and 20 °C for GE119 genotype pollens, and +4 °C for GK138 genotype pollens were found to be effective. In terms of pollen diameters, while the pollen of the Gemlik cultivar had a higher diameter than the others, the GK138 genotype had a low pollen diameter. However, pollen diameters were higher in olive pollens stored at -80 °C for 35 days. The study showed that Gemlik, Domat, and GE119 pollens could be stored acceptably at zero temperatures for 35 days. Aside from their contribution to artificial pollination studies, the obtained results are critical in terms of incompatibility evaluations and producing a solution to the pollination problem of genotypes that did not bloom at the same time.

In cross-pollination of cultivars, the effective pollination period and the amount of time pollen can stay alive can be very different from one another. In addition, thanks to pollen retention, pollen from cultivars that are not already present in the region can provide great convenience to pollination efforts by preserving their viability and germination abilities. It also helps to save time during studies.

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