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***In vitro* Pollen Germination of Four Olive Cultivars (*Olea europaea* L.): Effect of Boric Acid and Storage**

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ABSTRACT

This work aimed to optimize the pollen germination of four olive cultivars Koroneiki, Frantoio, Manzanilla and Nabali, in order to use pollen resource in cross pollination assays. Pollen samples were subjected to 2 experiments to assess the effect of boric acid and storage treatments on the germination fertility (viability, germination and tube growth). First, pollen of each cultivar was exposed to boric acid at 0 and 100 ppm added to an agarized medium. For storage, pollen grains were conserved in freezer (-20°C), in refrigerator (+10°C) and at room temperature (25°C; control) during 0, 1, 6 and 12 months. The results showed that the addition of 100 ppm boric acid to the culture medium not only increased the pollen germination by 12-24.9% according to the cultivars but also resulted in an increased tube length after 5 h instead of 9 h in control (without boric acid). Pollen of all cultivars showed greater tolerance for low temperature storage -20 and 10°C and not for high temperature (25°C). Increasing storage period of pollen grains from 1 to 12 months, resulted in a decrease in those parameters in all cultivars tested, these reductions were evident after one month of storage. Pollen death occurred rapidly after 1 month when held at room temperature (25°C) while for temperatures storage of -20 and +10°C, pollen death occurred after 6 months. These pollen characters indicate a potential for using these pollen grains as pollinator for self-incompatible olive cultivars found in Al-Jouf conditions.

Key words: Boric acid BA, pollen germination, *Olea europaea* L., pollen conservation, meteorological data

INTRODUCTION

Olive trees in the Kingdom of Saudi Arabia are produced mainly in the north, in 3 of a total 13 provinces which are Al-Jouf, Hail and Tabuk. Agriculture in these regions represents 244.920 ha (30,36%) of the total area planted with fruit trees (according to the statistics of the MoEP (2010). In 2011, there are about 23245 ha of olive trees in KSA, out of which 22495 ha (96.77%) in Al-Jouf, Hail and Tabouk provinces and 95% were at the young age of fruiting (less than 10 years) (Mehri *et al.*, 2013a).

It's well known that pollen has a direct effect in fertilizing process in plant breeding (Androulakis and Loupassaki, 1990; Ateyyeh *et al.*, 2000). Their effect has been studied on *in vitro* and *in vivo* germination of self incompatible pollen in olive (Mehri *et al.*, 2003), in apple (Montalti and Filiti, 1984) and in walnut (Luza and Polito, 1985). In olive, pollen grains were very useful providing a source of pollen for improved yield by cross pollination (Bradley *et al.*, 1961; Eassa *et al.*, 2011) and for use in research program (Moutier, 2002; Mehri *et al.*, 2003; Cuevas and

Polito, 2004; Mehri *et al.*, 2007). Also, pollen grains have been used to examine the effectiveness of insecticides and bio-insecticides sprayed during flowering or added to germination media on the germination capacity of olive pollen (Mehri *et al.*, 2007). Several papers have studied the important role of meteorological parameters in the variation of pollen fertility, on olive flowering (Lavee *et al.*, 2002; Galan *et al.*, 2001a), pollen dispersion (Galan *et al.*, 2004; Vazquez *et al.*, 2003), airborne pollen concentration (Galan *et al.*, 2001b; Vazquez *et al.*, 2003) and *in vitro* pollen germination (Galan *et al.*, 2001a, 2005).

In order to improve the pollen capacity and to promote the fertilization process in olive trees, many substances have been used as boron added to germination medium or sprayed on the trees (Viti *et al.*, 1990) and pistil extract *in vitro* (Fernandez-Escobar *et al.*, 1983). Also, the effect of boron on trees has been studied on olive flowering and fruit set (Perica *et al.*, 2001, 2002; Desouky *et al.*, 2009), on oil content and oil quality (Desouky *et al.*, 2009). The use of Boron foliar application at 100 ppm and calcium at 2%, alone or in different combinations at full bloom and 15 days after, resulted in improved fruit set, oil content and oil quality (Desouky *et al.*, 2009).

Pollen storage is considered as the most efficient method to overcome barriers to hybridization between plants flowering at different time or growing in different regions. In olive the use of stored pollen is necessary to pollinate a flower successfully in pollination assays. According to Griggs *et al.* (1975), Fernandez-Escobar *et al.* (1983), Polito *et al.* (2003) the development of suitable methods for olive pollen storage and preservation can offer possibilities for long time conservation. Long term storage of pollen has been achieved among different storage methods and cold storage proved to be the most economical and widely used method for preserving pollen. Ganeshan and Alexander (1991) and Alba *et al.* (2011) suggested the use of the cryo-preservation of pollen using liquid nitrogen at -196°C. Several studies have been published on the determination the optimal conditions to maintain the pollen viability during a long time. The main factors affecting the viability and germination capacities of stored pollen are temperature and relative humidity. Pinney and Polito (1990) obtained good results when olive pollen was stored at 28-33% RH at -20°C.

The present study focuses on improving the pollen fertility: viability, germination and tube growth (1) by using boric acid at 0 and 100 ppm in the pollen germination medium and (2) by testing three storage methods (at -20; +10 and 25°) and four periods (from 0, 1, 6 and 12 months) of four olive cultivars grown in Al-Jouf, northern part of Kingdom of Saudi Arabia.

MATERIAL AND METHODS

The olive cultivars studied are Koroneiki, Frantoio, Manzanille and Nabali. They are of Greek, Italian, Spanish and Jordanian origin, respectively and constitute the main olive cultivars diffused in Al-Jouf (KSA). They are introduced in KSA without any study on their morphological characteristics. Recently, a preliminary trial showed a variation in pollination efficiency and yield of these cultivars in the region of Al-Jouf (Mehri *et al.*, 2013a).

Frantoio: Also Frantoiano, Correglio, Razzo is cultivated in Toscane (Italy). Olives are medium sized and produced an oil of high quality. The cultivar shows a high tolerance to cold. Its oil is very fruity, aromatic and herbaceous, medium bitterness and stability, strongly pungent.

Koroneiki: Cultivated in Greece (Péloponnèse, Zante, Crète, Samos). Olives of small size are black at ripeness. Oil highly appreciated in Crete is strongly fruity, herbaceous and very stable, mild bitterness and pungency.

Manzanille: Also Manzanilla is a Spanish cultivar (Andalousie). Olives are medium-sized with a small pit from which the flesh separates easily. Fruits rounded, green or black are used for oil and table olives. Oil of Manzanille is fruity, aromatic and herbaceous, medium bitterness and stability, strongly pungent.

Nabali mohassen: Synonyms Improved Nabali, Muhassan, Nabali, Nabali Mohassan, Raseei, Rasie, Rsa'si, Ras'i. It is a dual purpose cultivar largely diffused in the Middle East having high oil content (about 30%). The cultivar shows very high tolerance to drought and cold and has a low sensitivity to olive fly and medium tolerance to peacock eye spot.

Meteorological trends: The experiments were carried out during 2010 at the olive research unit of Al-Jouf, KSA, which is located at an altitude of 684 m above sea level, latitude 29.8° N and longitude 40,1° E. As the environmental conditions of olive trees and of flower samplings are important factors for pollen germination, the meteorological data were collected in the airport station of Al-Jouf region (KSA), located 3 km sec⁻¹ from the sampling site. Climate Al-Jouf-Historical weather (<http://www.tutiempo.net/en/Climate/Al-Jouf/403610.htm>). The parameters concerned the monthly means of the average maximum and minimum air temperature, relative humidity, wind and rainfall.

Pollen collection: Pollen of each cultivar was collected in spring 2010 from flowers having the same physiological stage (anthesis) from various inflorescences. They were selected and harvested just before opening (phenological stage 60, based on BBCH scale of Sanz-Cortes *et al.* (2002)). They were wrapped in Zip lock bags to prevent dehydration during transportation and left in the laboratory at 25°C room temperature during 24 h. The fresh pollen grains were collected and immediately kept in plastic vials, sealed with parafilm.

Boron assays: In order to assess the influence of boric acid in pollen germination of the four olive cultivars, fresh pollen grains were subjected to *in vitro* germination tests, with 2 concentrations of boric acid. The control consists of fresh pollen grains of each cultivar, cultivated on a basal medium, which is an agarized medium containing 0.7% agar, 20% sucrose at pH= 5. To test the effect of boron on pollen fertility (pollen viability, germination and tube growth), 100 ppm of boric acid was added to the culture medium before autoclaving. Cultures were held at 25°C in dark for 24-48 h.

Storage tests: To assess pollen sensitivity to storage temperature and period, fresh pollen grains obtained were divided into 3 lots: stored in freezer at -20°C, in refrigerator at +10°C and without conservation at room temperature 25°C for four storage periods of 0, 1, 6 and 12 months. The control consists of pollen leaved in laboratory temperature at 25°C. After one, 6 and 12 months of storage, pollen was removed from the freezer (-20°C) and the refrigerator (+10°C) and leaved to room temperature during 12 h before germination. Pollen was then germinated in Petri dishes containing 10 mL of the control agar solidified medium with 100 ppm boric acid. Pollen grains of each cultivar and each treatment were sprinkled in fine layer on the surface of the medium.

Pollen viability, germination and tube length: For viability tests, freshly collected and conserved pollen (after 1, 6 and 12 months of storage) of all the cultivars were tested following the procedure of Martoja and Martoja-Pierson (1967) by staining with aceto-carmin. The evaluation

of germination and tube growth of stored and fresh pollen was assessed following optimization of the best medium and which contain 0.7% agar, 20% sucrose and 100 ppm of boric acid, at pH= 5. Fresh and stored pollen were incubated at 25°C and observed for germination and tube growth under inverted microscope after 3, 5, 12, 24 and 48 h of culture on pollen grains of each cultivar chosen at random from various locations in the pollen sample. Germination rate and tube length were determined using two replicates of approximately 100 grains (Pinney and Polito, 1990). Pollen grains must produce tubes equal to at least twice the diameter of pollen grains to be counted as germinated pollen while burst pollen was not counted as germinated (Stanley and Linskens, 1983).

Statistical data: The effect of boric acid, storage period and storage temperature on viability, germination and tube growth of four olive cultivars was analyzed by analysis of variance. Data obtained were tested to determine significant differences between cultivars and storage methods on pollen viability, germination and tube length. Standard errors of each mean were calculated and presented in tables.

RESULTS AND DISCUSSION

Meteorological data: The changes in monthly mean maximum (TM), minimum air temperature (Tm), relative humidity (RH%), rainfall and wind during flowering period of the four olive cultivars (from February to June 2010) are given in Table 1. It is evident from the meteorological data that under field conditions in Al-Jouf, the weather was characterized by high air temperature and low relative humidity (between 30-14.3%). The monthly mean maximum air temperature ranged between 23-39.6°C and the monthly mean minimum air temperature ranged between 9.6-24.8°C respectively from February to June 2010. Under these conditions, olive trees had to withstand more severe drought conditions with a total of 19.80 mm of precipitation distributed only in April and May 2010.

Effect of boric acid on pollen fertility

Germination and pollen tube growth: Table 2 reports the effect of boric acid (0 and 100 ppm) added in basal medium on the average percentage of pollen germination and tube length of four olive cultivars: Koroneiki, Frantoio, Manzanille and Nabali. An analysis of variance demonstrated that there is a significant difference in pollen germination and in cultivars among treatments. In absence of boric acid, pollen of all cultivars germinated, but with lower values, related to the cultivars. In control, viability, germination rate and pollen tube length were significantly higher for 'Koroneiki' pollen (81.3%, 44.59% and 392.9 µm) and lower for Nabali (50.6%, 24.8% and

Table 1: Monthly means of maximum and minimum temperature, relative humidity, rainfall and wind speed in Al-Jouf area of KSA from February to June 2010

	Mean temperature T (°C)	Maximum temperature TM (°C)	Minimum temperature Tm (°C)	Relative humidity RH (%)	Rainfall amount P (mm)	Mean wind speed V (km h ⁻¹)
February	16.3	23.0	9.6	30.0	2.03	15.5
March	20.1	27.0	12.8	27.0	0.00	15.9
April	24.3	31.2	16.8	23.0	11.69	14.9
May	29.1	35.7	21.3	17.0	5.08	14.5
June	32.7	39.6	24.8	14.3	0.00	16.4

Flowering of the four cultivars occurs between mid March and end May

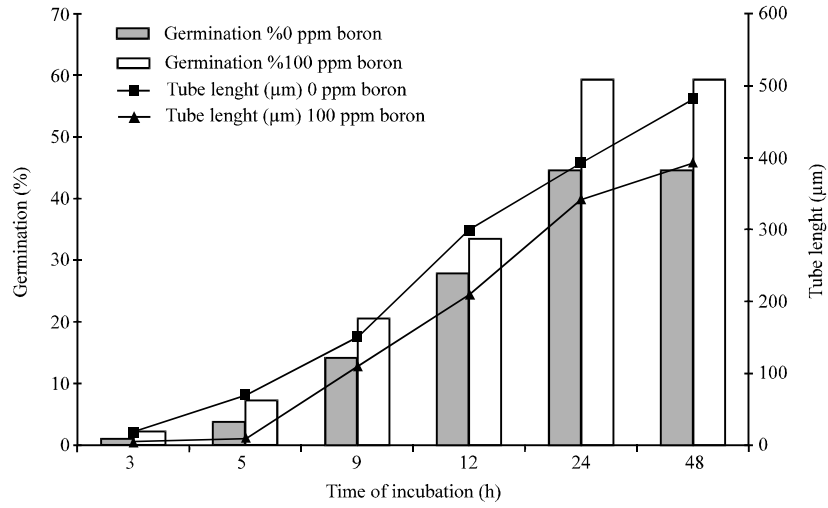


Fig. 1: Effect of boric acid added to the pollen germination medium on germination rate and tube growth of koroneiki olive cultivar

Table 2: Effect of boric acid at 0 and 100 ppm added in medium containing 0.7% agar, 20% sucrose, at pH= 5, on the pollen germination capacity of four olive cultivars incubated at 25°C during 24-48 h in dark

Cultivars	Boric acid concentration (ppm)	Pollen viability (%)	Pollen germination (%)	Increase of pollen germination	Pollen tube length (μm)	Increase of pollen tubes
Nabali	0	50.65±3.5	14.25±1.9	12.6%	144.9±7.9	48.5%
	100	-	24.81±1.09	281.3±6.09		
Frantoio	0	67.11±2.77	37.18±4.06	12.1%	347.8±5.7	33%
	100	-	42.26±6.1	519.1±7.6		
Manzanille	0	69.78±5.09	27.33±3.7	19.4%	263.6±2.8	46.4%
	100	-	33.90±2.9	491.7±2.91		
Koroneiki	0	81.32±3.49	44.59±3.9	24.9%	392.9±4.6	25%
	100	-	59.31±4.2	523.5±4.3		

Values are taken as Mean±Standard error

281.3 μm) (Table 2). Basal medium supplement with 100 ppm boric acid allowed higher mean percentage of germination and higher pollen tube development in all the olive cultivars tested but the values are also dependant to the cultivars. Pollen germination *in vitro* averaged 24.81, 42.26, 33.9 and 59.31% in Nabali, Frantoio, Manzanille and Koroneiki cultivars, respectively. Pollen tube length followed the same trend as it was respectively 281.3; 519.1; 491.7 and 523.5 μm.

The addition of boric acid at 100 ppm to the medium, leads to an increase in germination percentage from 12.1-24.9%, high increase of tube length from 25-48.5% according to the cultivar. It is clear that 100 ppm of boric acid is more efficient in Koroneiki and Manzanille cvs with an increase in germination while in Nabali and Manzanille cvs, boric acid was more efficient in tube length with an increase of 48.5 and 46.4%, respectively. No significant differences were found for the increase of pollen germination in Nabali and Frantoio cultivars, (12%) (Table 2). The Nabali cv gave least results with an increase of 12% in pollen germination, but the tube length reached after 48 h of incubation 144.9 μm in absence of boric acid and 281.3 μm in presence of boric acid. The highest pollen viability and germination in the conditions of Al-Jouf was enregistered in

Koroneiki cultivar (81.32 and 59.31%, respectively). Eassa *et al.* (2011) found also a high viability rate of Koroneiki pollen in the conditions of Cairo-Alexandria (from 83.13-91.19%).

In this study, boric acid enhanced pollen germination and mainly growth of pollen tube *in vitro*, according to the cultivars. It's in agreement with results of Pinney and Polito (1990) where the concentration of 50 ppm boric acid proved to be more efficient in Pendolino cv and 100 ppm gave better results in Moraiolo cv. While in Leccino and Frantoio cvs, the presence of 100 ppm BA in germination media was ineffective. *In vitro*, Ateyyeh *et al.* (2000) have used 50 ppm citric acid in germination medium of olive pollen of Nabali Baladi cultivar while Pinney and Polito (1990) tested pollen germination of Manzanillo, Ascolana and Mission cultivars with 100 ppm boric acid and 60 ppm tetracycline.

Time of germination process: Figure 1 illustrated the individual performance of Koroneiki cultivar during pollen germination in absence or in presence of boric acid. After 48 h of germination, the germinative capacity was lower in control (0 ppm boric acid) with a slow growth of the pollen tube. The beginning of the germination process occurs after 9 h of incubation. In presence of boric acid, Koroneiki pollen start to germinate earlier and presented a faster pollen tube growth, germination and tube growth were enhanced mainly during the first hours of incubation. After 3 h incubation, the mean germination percentage in the control was 0.9% and tube growth was 5 μm . In presence of bore, it was 2.1% and 17.8 μm , respectively.

Observations made after five hours of incubation showed high number of germinated grains with good pollen tube elongation. After 48 h at 25°C, the optimum germination percentage and tube length was 44.7% and 392.5 μm , respectively in control, while it was 59.4% and 481.09 μm in presence of boric acid at 100 ppm. The addition of 100 ppm boric acid not only increased the germination rate (by 44.59-59.31%) but also resulted in increasing tube length earlier than control (0 ppm) by 392.9-523.5 μm (5 h instead of 9 h in control), it induced an earlier germination process.

This beneficial effect of boric acid in *in vitro* germination under controlled conditions was similar to these recorded by Acar *et al.* (2010) with pistachio *in vivo* pollen germination and Viti *et al.* (1990) with olive pollen and in the field conditions. The latest underlined the positive effect of boric acid on olive fertilization assays and on the process of pollen tube growth in self pollinated Leccino cv which is known to be high self-incompatible. Desouky *et al.* (2009) improved olive fruit set, oil content and oil quality of three olive cultivars by spraying, during flowering, 100 ppm boric acid (H_3BO_3) and 2% calcium. Spinardi and Bassi (2012) signaled the importance of cross-pollination and boron on the olive fertility.

Pollen tube morphology: Pollen tubes developed in control medium differed morphologically from pollen tubes cultured in presence of boric acid. Lack of boron inhibited pollen tube growth and caused morphological abnormalities as swelling at the tip of pollen tube and high proportion of pollen grains with irregular shape. In presence of boric acid in the cultured medium, pollen tube had a normal length and shape showing straight and long tubes. The observed inhibition and morphological alterations in pollen tubes by deficiency of boron is consistent with the result of previous studies of Wang *et al.* (2003) on Picea and Viti *et al.* (1990) on olive. Similar abnormalities have been reported for olive pollen grains and tubes exposed *in vitro* germination to insecticides (Mehri *et al.*, 2007). Hu and Brown (1994) explained that boron deficiency greatly reduces cell wall plastic extensibility in plants.

This study has reported that boron added in the form of boric acid is essential for the *in vitro* pollen germination and tube formation and its deficiency results in poor pollen germination and reduced pollen tube growth. This result is of great interest on olive pollen ability, because the greater germination coupled with greater tube length in presence of bore make olive pollen more effective in pollination by increasing fertilization. Further tests have to be conducted by increasing the concentration range of boric acid *in vitro* and *in vivo* pollen germination under Al-Jouf conditions.

Effect of storage treatment on pollen viability, germination and tube growth: The influence of different storage methods on pollen viability, germination and tube growth of four olive cultivars were investigated in this study. Pollen grains of each cultivar were stored under the 3 storage temperatures: - 20, +10 and +25°C and germinated at storage periods of 0, 1, 6 and 12 months. Germination was determined at 25°C and pH=5 in an agarized medium (0.7% agar, 20% sucrose and 100 ppm boric acid). The control consists of fresh pollen grains of all the cultivars stored at 25°C and incubated at 25°C on the same basal medium. Pollen viability, germination rate and tube length were determined after 3, 5, 12, 24 and 48 h.

The cultivar x storage temperature x storage period interaction was significant, indicating that cultivars differed in their response to storage treatment on pollen viability, germination and tube elongation. The results obtained indicate that germinability of pollen varies according to the cultivars and fresh pollen of all studied olive cultivars gave significantly higher viability and germination rates and higher tube length compared to the stored pollen.

Control: In control, noticeable differences in viability and germination rates and tube elongation were found between pollen of the different cultivars (Table 2). Fresh pollen of Koroneiki gave higher viability (81.3%) and germination (59.3%) than the other olive cultivars. Nabali cultivar showed the least values (50.6 and 24.8%) and Manzanille was the second in pollen viability (69.7%). Also, tube length varies according to the cultivar, it was lower in Nabali (281.3 µm) and higher in Frantoio, Manzanille and Koroneiki cultivars (519.1, 491.7 and 523.5 µm, respectively). Previous studies conducted by Mehri *et al.* (2013b) confirmed the low pollen fertility of Nabali cultivar under the same conditions of Al-Jouf province with a viability rate of 51.21% and germination rate of 18.17%. For all the cultivars, the pollen germination *in vitro* was always lower compared to pollen viability. This can be due to the effect of several variables which are difficult to control such as pollen density, optimum culture media and environmental exigence of each cultivar.

Storage temperature: It is clear from data in Table 2 that the three different temperatures (-20, +10 and 25°C) at 4 storage periods (0, 1, 6 and 12 months) influenced the pollen viability, germination and tube growth.

When stored at +10°C, the germination rate decreased more weakly compared to 25°C and became 9.01, 3.7, 7.6 and 15.2% after 180 days of storage for respectively Nabali, Frantoio, Manzanille and Koroneiki cvs. After one year of storage at +10°C (Table 2), the germination rates decreased, in all the cultivars tested to less than 3%.

Pollen stored at -20°C showed good germination but as the time proceeds, the viability and germination percentages gradually decrease. At -20°C, the germination rates of fresh pollen grains were 24.8, 42.2, 33.9 and 59.3% for Nabali, Frantoio, Manzanille and Koroneiki cvs, respectively.

After 30 days of storage, these rates become 13.1, 31.3, 27.9 and 28.6%, respectively and decreased more to 2.6, 11.6, 18.2 and 7.9% after 6 months of storage (Table 2).

When held at ambient conditions maintained at $25\pm 2^{\circ}\text{C}$, viability, germination and tube growth were highly reduced compared to -20 and 10°C . It resulted in the lowest germination and tube growth rates for all four cultivars. The ability of pollen to germinate declined more rapidly, pollen grains didn't germinate after being kept at 25°C ambient conditions for more than one month. The percentage germination decreased over one-half during the first month. Period storage of 6-12 months and storage temperature of 25°C were the most inhibitory on the germination of all pollen cultivars (Table 2). Storage at this temperature resulted in complete loss of pollen viability and germination by 6 months for Nabali cv (9.03% and 1.8%) and 12 months for Frantoio cv (0.37%, 0.12%), for Manzanille cv (1.13%, 0.9%) and Koroneiki cv (3.9%, 1.75%). This loss in viability and germination was associated with a decrease in pollen tube length *in vitro* in all the olive cultivars tested. Tube lengths were highly inhibited when pollen were conserved more than 1 month. In contrast, germination didn't differ when stored 6-12 months.

Our results revealed that temperature of 25°C proved to be lethal for the pollen of all cultivars studied after one month of storage. Pollen have been stored satisfactory at -20 and 10°C from long time and can be used in olive breeding programme and artificial pollination management to insure high fruit set on olive cultivars planted in Al-Jouf conditions. All these results can play a very important role in solving the compatibility problems in olive and in determining the successful adaptation of olive cultivars.

Storage period: In all olive cultivars tested, the mean percentage viability and germination dropped with increasing the period of storage and became lowest at 12 months of storage. Germination ability of stored pollen for more than 1 month was highly reduced (Table 2), pollen lost rapidly the capacity to germinate with lowest germination in any of cultivars after 6-12 months and this, at any storage temperature of -20 and 10°C . The optimum storage temperature was $+10^{\circ}\text{C}$ in the refrigerator for at least one month for all the olive cultivars. Significant pollen tube length was found between pollen of the four olive cultivars studied; it decreased with increasing storage period of pollen grains from one to 12 months suggesting that storage temperature had a significant detrimental effect on the growth of pollen tube. Germination of pollen stored for one year was highly reduced less than 3%. At this date, pollen lost rapidly their capacity of germination with no germination in all cultivars from 6 months at 25°C . Over the storage period, pollen showed little loss in viability when stored either at -20 and $+10^{\circ}\text{C}$.

In this study, temperature of 25°C is considered as optimum temperature for pollen germination of the studied cultivars; in contrast, it has been showed to be lethal for pollen storage. This temperature represents the average temperature for March, April and May in Al-Jouf. These findings confirm the results of germination capacity of stored pollen of olive (Pinney and Polito, 1990; Koubouris *et al.*, 2009) and concur with those of Stanley and Linskens (1983) where pollen stored at low temperature presented better germination capacity than high temperature. Storage pollen at 25°C in the conditions of room labor without any relative humidity control can be the cause of the high decrease in germination capacities over storage period.

High rates of pollen viability, germination and tube length are maintained over the first month of storage with all cultivars, at -20 , $+10$ and $+25^{\circ}\text{C}$. In olive trees, this period may be sufficient to the cross pollination assays using this pollen as pollinator in the same year as suggested by Cuevas *et al.* (2001). Regarding the results, it is important to establish a bank germplasm with

stored pollen of different olive cultivars to be used for more than a season work in pollination and in breeding programs. Also preserving the viability and germination of pollen permit a considerable saving in time because olive cultivars blossom at different periods and are cultivated in different localities which make difficult to collect pollen.

Olive cultivars: All cultivars were significantly different with respect to their pollen fertility following the three storage periods. This difference observed in their sensibility to storage temperature was -20°C for Frantoio and Manzanille cvs. and +10°C for Koroneiki and Nabali cvs, while all the studied cultivars were sensitive at 25°C. This confirms the genotype-treatment interaction observed in olive by Fernandez-Escobar *et al.* (1983). It may be based on relative tolerance to low and high temperature as pollen showed greater tolerance for low temperature storages (-20 and +10°C) and not for high temperature of 25°C. Kalkar and Neha (2012) explained that esterase enzyme contained in pollen grains is quite active at low temperature and the integrity of plasma membrane of pollen grains was more than pollen stored at room temperature and +4°C.

Pollen viability was observed to vary between cultivars confirming the results of Wu *et al.* (2002) with a low of 14% in Pendolino to a high of 79% in Frantoio; therefore, multi-year data must be obtained in order to observe the effect of changes in vitality on the ability of each cultivar to act as a pollen donor in cross pollination assays.

Pollen of Koroneiki cv gave the highest values under Al-Jouf conditions, this was with fresh pollen or stored pollen. Also, the treatment temperature of 25°C prevented significantly pollen germination in Nabali, Frantoio and Manzanille cultivars while Koroneiki cv was less affected with an average of 26.6% compared to the control (59.3%). These observations agree with conclusions of Chartzoulakis *et al.* (1999) who confirmed the ability of Koroneiki cv to maintain higher photosynthetic rates under water stress conditions.

The difference found on pollen viability and germination rates in all cultivars tested (Table 2) can be due to genotypes, pollen harvesting conditions and transport to the laboratory. Al-Jouf conditions during the flowering period (2010) characterized low relative humidity and field conditions can play an important role and influenced pollen fertility. Our results agree with previous studies in olive (Koubouris *et al.*, 2009) where a short exposure of olive pollen at high temperature combined with low RH before *in vitro* culture had detrimental effect on pollen ability of all studied olive cultivars.

Climatic conditions: Climatic conditions of Al-Jouf may explain the various performances in pollen germination of the four cultivars obtained in this study. This region is characterized by high natural variability with the presence of important limiting factors. Olive trees were directly exposed at environmental extremes where pollen is subjected to high temperature and drought conditions that may affect its germination ability. Flowering period of Al-Jouf in 2010 (February to June) was mostly dry with increasing mean temperature and with small amount of rainfall occurring on only a few days. The maximum temperatures ranged from 23-39.6°C with an average of 21°C during flowering period, which is suitable for olive pollen tube growth according to Cuevas *et al.* (1994a, b).

Several studies have reported that environmental conditions can affect the process of pollination in olive (Bradley *et al.*, 1961; Hartmann and Opitz, 1966; Orlandi *et al.*, 2003, 2010). Temperature was considered to be one of the most influencing variables for the flowering process (Perez-Lopez *et al.*, 2008) and dry season causes water stress (Correia *et al.*, 1992). Martin (1994)

considers that low temperatures have been found to reduce pollen tube growth in olive, which is unable to reach the embryo sac before it degenerates. While, high temperatures result in faster growth of pollen tubes (Griggs *et al.*, 1975). Fernandez-Escobar *et al.* (1983) and Cuevas *et al.* (1994a) observed better pollen tube growth at 25°C than at 30-35°C. Orlandi *et al.* (2003, 2010) confirmed the strong relationship between olive flowering period and spring temperature trends in Italy and observed that temperature during March, April and May, was the parameter most related to flowering date. On most days, the maximum temperature reached at Al-Jouf is higher than 30°C, which may affect pollen tube growth. High temperature can cause dehydration on pollen grains and alternate their biochemical and chemical constituents causing damage to the pollen cytoplasm and lead to the death of pollen as suggested by Pacini (1996). According to Ayerza and Sibbett (2001) high air temperatures appear to have nefast effect on olive flowering and this effect was more pronounced on tube growth. These results established the necessity of studying the impact of these extreme conditions for more than several years on pollination to study pollen-pistil incompatibility in relation to climatic factors. Wind speed and direction during bloom time are other environmental factors to consider in Al-Jouf region because olives are wind pollinated.

CONCLUSION

The present study was undertaken to evaluate the fertility differences in the pollen grains of four olive cultivars in presence of boric acid and under three storage methods. Increasing storage period (from 0 to 12 months) and storage temperature (from -20 to +25°C) resulted in reducing pollen germination, viability and tube length in all the cultivars. While using boric acid in germination medium provided stimulation of pollen germination of all the olive cultivars studied and allowed higher mean percentage of germination and higher pollen tube development. These results are important for olive breeding program because the pollen storage of the four pollen cultivars can be used to further investigate the fertility process in olive by cross pollination assays.

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