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Assessment of olive genotypes towards phenology, pollen viability and germination in Pakistan

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# Abstract

Pollen viability and germination tests are consider key factors for final productivity. Hence characterization of pollen and phenological aspects of eighteen olive cultivars were studied under an experiment carried out at Barani Agricultural Research Institute, Chakwal, Pakistan. Pollen viability was tested through Acetocarmine and germination was analyzed in the culture media having water, boric acid, sucrose and agar. Results depicted that the maximum number of panicles were recorded in variety Earlik (27.90) and minimum in variety Ottobratica. In general more number of panicles was recorded in 2018 in all most all the varieties as compared to 2017. Highest numbers of flower per branch were observed in variety Ottobratica. Maximum final fruit set percentage was recorded in variety Moraiolo (4.23 %) and Coratina (3.70 %) in first year of study while Coratina (3.75 %) and Frantoio (3.59 %) depicted highest fruit set percentage in second year of study. Maximum pollen viability (79.18% & 53.08) was recorded by in Variety Gemlik while minimum values were recorded in Variety Leccino (27.16 % & 47.62 %) in both the years 2017 & 2018 respectively. The germination rate varied among all the varieties. Varieties Ottobratica and Gemlik showed maximum value of pollen germination rate during the study year while minimum rate was recorded again in variety Leccino.

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#### Introduction

Olive (Olea europaea L.) is an evergreen tree and prominent species of whole Mediterranean countries. Its cultivation can be traced back since ancient times and is also mentioned in the Holy Quran, Hebrew and the Christian Bibles (Flaishman et al., 2008). The olive fruit is a famous fruit tree worldwide for its oil and nutritional benefits. Successful fertilization and fruitlet persistence is directly dependent upon flower quality (Martins et al., 2006) which also predict pistil abortion rate, embrosac development, and receptivity of stigma, pollen viability and nitrogen and carbohydrate contents (Cuevas et al., 1994; Moreno-Aloaas et al., 2018). Large ovaries are associated with more fruit set. (Cuevas and Polito, 2004.) Flower quality is better in off year than on year production (Mazzeo et al., 2014). The length of inflorescence (3-8 cm) and number of inflorescence per branch and number of flower (15-30) per inflorescence varies with the cultivar (Martin and Sibbett, 2005). These characteristic may vary in each year, plant, branch, inflorescence (Brooks, 1948; Cuevas et al., 1994; Lavee et al., 1996; Lavee et al., 2002; Reale et al., 2006). The perfect or staminate flower may vary with the position of inflorescence (Bouranis et al., 1999; Dimassi et al., 1999; Ateyyeh et al., 2000; Cuevas and Polito, 2004).

Most important features in olive fruit set are selfincompatibility, self-fertility and abnormalities in morphological traits. Various factor of sterility are present in olive like more or less related species which are responsible for productivity. Olive trees with good quality and quantity of viable pollens is essential for a good pollination. As a whole, there exists a linear relation between viability and germination capacity of pollen and germination capability in many fruit species (Stanley and Linskens, 1974). Both viability and germination depends on various factors like genotype, nutrition conditions, and biotic and a biotic factors (Khan and Perveen, 2008).Poor pollen viability and germination percentage cause less fecundation in olive (Bini, 1984; Tombesi, 2013). Pollen viability is a genetic trait (Vuletin-Selak et al., 2014) and estimated by various techniques and one of the most important is staining with acetocarmine jelly (Radford *et al.,* 1974).

Pollen germination is another important factor in olive fruit production. Growth of pollen tube in In vitro condition should be check to assess the pollen grain germination capability. Temperature and relative humidity has strong influence on pollen tube growth along with the humidity and growing media.

There is a huge difference in pollen germination on stigma in open field condition and in vitro growth (Heslop-Harrison and Heslop-Harrison, 1981; Pinney and Polito, 1990; Kovacs and Barnabas, 1997). *In vitro* Pollen tube germination is very sensitive to growing media. In present conditions media is considered to be suitable having water, boric acid, sucrose and agar (Pinney and Polito, 1990) and citric acid (Al-Dehadhehetal *et al.*, 2004).

The media containing 20% sucrose is optimal and more than 30 % cause inhibition of pollen tube growth (Lavee *et al.*, 1985). Pollen ability, germination percentage and pollination of 18 olive cultivars were evaluated. It is believed that pollen ability is an important tool for pollination process for mono orchard cultivation. Major significant differences among cultivars for pollen abilities were listed with variation in characteristic with reference to time (Shemer *et al.*, 2014).

Olive cultivation on commercial scale has no long history in Pakistan. But during the last ten years olive plantation has gained popularity because of its great socio-economic importance. More than 4494.36 hectares have been brought under olive cultivation in Pothwar region and 2,800 acre (280,004 plants) was cultivated other than Pothwar region under Federal and provincial Government, Pakistan (PARC, 2018; BARI, 2019).

A major aim was to examine phenology, pollen viability and germinating capacity of pollens of eighteen exotic olives for identifying the most suitable genotype for newly olive industry establishment.

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#### Material and methods

### Location with metrological data

The experiment was conducted for two consecutive years in 2017 and 2018 at Barani Agricultural Research Institute, (BARI) Chakwal, Pakistan (32º 92'82 N and 72º 7201 E) located at height of 575 m above the sea level. The average precipitation in last ten years was 772 mm with 36-38 °C and 1.67 °C maximum and minimum average temperatures in summer (June) and winter (January) respectively. Maximum rain fall received 408 mm in monsoon season ranges from July to September (Table 1). During the two years of study period (2017 & 2018), average rainfall during the year was 786 mm with average maximum temperature in June (36.6°C) and minimum temperature in January (2.55°C). The weather condition during the study period is mentioned in Fig. 1.

# Plant material

Plants (age of 8 to 10 years) of 18 exotic olive cultivars were selected with uniform canopy planted in sandy loam soil (organic matter less than 1% and pH of 8.1) with planting geometry of 6m × 6m. Name of varieties in detail has been presented in Table 2.

### Phenological parameters

Varietal behavior towards phenological parameter was investigated. Number of panicle/branch, number of flower/branch, staminate, pistilate flower and fruit set percentage were calculated from four randomly selected branches from each side of canopy (Iqbal *et al.,* 2019).

#### Pollen viability and germination

Pollen viability and germination was investigated for each variety for two years. For this purpose anthers of ten flower buds were collected at full development stage at the time of late afternoon with the help of forceps (Sanz-Cortés *et al.*, 2002). For efficient pollen grain collection, anthers were stored in uncapped petri dishes at room temperature 27° for 12-16 hours in the absence of all types of lights (Ramos *et al.*, 2008). Then pollens of each cultivar were inoculated over the surface of the medium of petri dishes having culture medium of 20 mL with the help of a brush for uniform distribution. After 72 h the pollen germination percentage were counted with the help of 10x objective lens microscope. The germination standard that consider for pollen grains was if the size of the pollen tube developed greater than double of the diameter of original pollen grain (Chagas *et al.*, 2010).

#### Statistical analysis

The experiment was laid out according to Randomized Complete Block Design with three replications. The data for eighteen varieties were analyzed to determine the phenological parameters, pollen viability and germination capacity through statistical software XLSTAT, 2014 (v.5.03) and least significant difference (LSD) method and assessed at the 5% significance level (SAS, 2005).

# **Results and discussion**

#### Number of panicles

The data regarding number of panicle per shoot was presented in Table 3. It reflected statistical significant variation among all varieties in both the years. Average number of panicle per branch ranged from 12 to 28 in 2017. The maximum value was recorded for the variety Earlik (27.90) followed by Manzanilla (23.88). The minimum value was recorded for the variety Ottobratica (8.67) nearby varieties of Chietina (9.40), Coratina (10.10) and Moraiolo (11.46). All other varieties were fall in between these ranges. In 2018, more number of panicle per branch was recorded in all most all the varieties. The maximum increase in number of panicle in 2018 were noted in varieties Ottobratica (8.75 to 23.38), Coratina (10.08 to 20.46), Chietina (9.42 to 20.92), Gemlik (13.75 to 19.5) and Nabali (14.63 to 20.13) in comparison of 2017. It could be concluded that all the varieties showed more number of panicles per branch in 2018 as compared to 2017 at both location. Olive tree bears flowers on the inflorescences which born in the axial of each leaf, which grow from the buds of the previous seasons growth. Approximately 15-30 flowers per inflorescence may be developed depending on the cultivars and growing conditions.

Months	Month wise average minimum	Month wise average maximum	Month wise average	
	temperature (°C)	temperature (°C)	rainfall (mm)	
January	1.67	16.61	23.52	
February	5.20	18.72	62.10	
March	10.19	25.09	60.32	
April	14.70	29.90	56.15	
May	19.80	36.20	45.15	
June	23.28	37.94	69.44	
July	23.91	34.97	197.34	
August	23.73	33.48	139.04	
September	21.00	32.96	65.34	
October	14.98	30.68	21.33	
November	7.45	24.54	6.37	
December	2.67	19.92	20.02	

Table 1. Average minimum,	maximum tem	perature (°C)	) and rainfall (	mm	) data of last ten y	vears.
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The variation in number of panicle per branch might be due to the quantity of carbohydrate reserves of the shoot which is considered to be an important for the development of the panicles (Jackson and Sweet, 1972). The other reasons might be the environmental conditions and fruit load of the previous year.

The major effect of on year and off year production may also contribute towards the production of panicle per branch.

## Number of flower per branch

Flowering is the most critical phase in the chronology of the annual cycle which governs the production. Number of inflorescence and number of flowers per inflorescence is considered to be varietal characteristics. The data regarding number of flowers per inflorescence was presented in Table 3. Average maximum number of flowers were recorded in variety Ottobratica (231.70) followed by Earlik (210.0) and Leccino (209.77).

Table 2. Name and origin of o	live varieties studied under Pothw	var agro-climatic condition of Pakistan.
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Variety Name	Origin	Variety Name	Origin	Variety Name	Origin
Earlik	Unknown	Gemlik	Turkey	Coratina	Italy
Manzanilla	Spain	Hamdi	Palestine	Pendolino	Italy
Frantoio	Spain	Nabali	Palestine	Leccino	Italy
Azerbaijan	Azerbaijan	FS-17	Italy	Chietina	Italy
Sevillano	Spain	Souri	Palestine	Correggiolo	Italy
Nocellera	Italy	Ottobratica	Italy	Moraiolo	Italy

The varieties Souri (73.47) and Sevillano (93.53) were ranked at bottom in 2017. All other varieties depicted the results in between mentioned two extremes. In 2018, again Ottobratica (335.96) ranked at top and variety Souri (107.80) remained at the bottom level. However in 2018, overall number of flowers per branch were found maximum in comparison of 2017. In the present study we concluded that varieties behaved differently regarding number of inflorescence and number of flower per inflorescence. The results are in accordance with many other studies which described that for a specific variety, number of inflorescence, number of flowers per inflorescence, pollen viability, fertility and ability of flower to be pollinated are a varietal characteristic (Kartas *et al*, 2015; Lavee *et al.*,2002; Martin and Sibbett, 2005).

concluded that flowers per inflorescence is highly

dependent upon on the genetic ability of the olive varieties however, many other authors believed that this is a stable and constant character for every determinate variety (Bini, 1984; Ghrissi, 2001; Lavee *et al.*, 2002).

Table 3. Olive varietal respo	nse towards phonologica	l characteristics during the study	years 2017 and 2018.
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Cultivars	s Number of panicles		Number of flowers		Staminate flower %		Pistilate flower %		Fruit set %	
	per	branch	per b	ranch						
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Earlik	27.9 a	28.34 a	210.0 ab	222.8 def	25.01 bcd	22.56 bcd	74.99 bcd	77.44 fgh	1.40 i	2.21 defg
Manzanilla	23.1 b	23.88 b	148.9 cdef	175.9 gh	25.37 bcd	19.96 cdefg	74.63 bcd	80.04 cdefg	2.64 de	2.78 cde
Frantoio	21.1 bc	23.38 bc	135.4 defg	163.6 hi	14.67 e	13.08 hi	85.33 a	86.92 ab	2.83 cd	3.59 ab
Azerbaijan	17.1 cdef	20.25 bcd	117.5 efg	159.3 hi	16.43 cde	14.45 ghi	83.57 abc	85.55 abc	2.55 def	2.62 cdef
Sevillano	12.5 ghi	17.42 defg	93.5 gh	143.1 i	23.20 bcde	21.61 bcdi	76.80 abcd	78.39 efgh	1.97 efghi	2.1 efg
Nocellera	13.2 fghi	16.34 efg	140 def	200.1 fg	26.10 bc	24.20 bc	73.90 cd	75.80 gh	1.50 hi	1.78 g
Gemlik	13.8 fgh	19.50 def	96.5 gh	161.7 hi	16.90 cde	11.93 i	83.10 abc	88.07 a	2.33 defg	2.88 bcd
Hamdi	18.8 bcd	19.420 def	123.9 defg	140.3 i	18.13 cde	17.15 defghi	81.87 abc	82.85 abcdef	2.97 bcd	3.22 abc
Nabali	14.6 defg	20.12 bcde	160.3 cde	240.2 cd	15.87 de	12.66 hi	84.13 ab	87.34 ab	3.63 abc	3.28 abc
FS-17	18.3 cde	19.63 cdef	128.2 defg	144.1 i	21.07 bcde	20.18 bcdef	78.93 abcd	79.82 defgh	2.23 defgh	2.86 bcd
Souri	13.9 efgh	17.34 defg	73.5 h	107.8 j	18.50cde	16.69 efghi	81.50 abc	83.31 abcde	2.64 de	3.27 abc
Ottobratica	8.75 i	17.46 defg	231.7 a	335.9 a	37.33 a	32.43 a	62.67 e	67.57 i	1.77 fghi	1.87 fg
Coratina	10.1	20.46 bcd	108.3 fgh	226.2 def	17.83 cde	15.66 fghi	82.17 abc	84.34 abcd	3.70 ab	3.75 a
Pendolino	17.5 cdef	18.83 def	185.4 bc	237.6 cde	28.40 ab	25.66 b	71.60 de	74.34 h	2.20 defgh	2.56 cdef
Leccino	14 efgh	16.42 efg	209.8 ab	260.5 bc	21.13 bcde	18.88 cdefg	78.87 abcd	81.12 cdefg	1.70 ghi	1.97 fg
Chietina	9.4 hi	20.92 bcd	135.6 defg	284.5 b	15.13 e	12.73 hi	84.87 a	87.27 ab	2.40 defg	2.92 bcd
Correggiolo	13.4 fgh	16.09 fg	158.8 cde	211.0 ef	21.47 bcde	17.48 defg	78.53 abcd	82.52 bcdef	1.70 ghi	2.22 defg
Moraiolo	11.5 ghi	14.25 g	164.5 cd	214.8 def	20.87 bcde	18.63 defg	79.13 abcd	81.37 cdef	4.23 a	3.22 abc

In Pakistan, we have less number of flowers as compared to the ideal Mediterranean climatic conditions. The possible reasons might be the high range of maximum temperature in summer with dry wind or incompletion of chilling hours to fulfill the requisite temperature for flowering. However we have the findings very similar to the findings of Raslan *et al.*, 2018 who found that varieties differ in number of flower per inflorescence and also varied with the season and genotype. The range given was almost found similar to our findings. It is well reported that minimum temperature of 2°C to 4°C and maximum temperature of 15.5 °C to 19 °C is ideal for flowering. Olive plants growth under constant temperature of 7°C or less produce flower (vernalization process) but fluctuation of warm condition support the related growth which may hinder in chilling effect (devernalization) (Denney and McEachern, 1983; Hartmann and Whisler, 1975). In our conditions summer temperature exceeded beyond 32 °C. Potential injury of high temperature in the region of Argentina and the regions of comparable environmental condition (like Pakistan) is reported in Non-Mediterranean climate so caution is necessary for expanding olive in Pakistan before in depth phenological study on olive germplasm. (Ayerza and Sibbett, 2001). However, in this scenario a model on the base of temperature is urgently needed for each phenological stage starts from bud induction to full bloom under specific environmental conditions.



**Fig. 1.** Daily maximum temperature (°C), minimum temperature (°C), rainfall (mm) for the month of 2017 and 2018 at BARI, Chakwal.

This type of scientific study will help in selection of wider range of varietal adaptation in variety of environmental conditions.

# Perfect and staminate flower (%)

A full bearing olive plant can produces more than 500,000 flowers per year. Olive produces two types of flowers i.e. perfect flowers and staminate flowers. The data regarding both types of flowers was collected for all the varieties as shown in Table 3. The statistical significant variation was found in between the years and varieties. In 2017, variety Ottobratica showed maximum staminate flowers (37.31 %) followed by Pendolino (28.39 %) while Frantoio ranked at the bottom with (14.66 %) of staminate flowers along with the variety Chietina (15.16 %).





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In 2018, overall varieties were found with less number of staminate flowers but Ottobratica remained at the top position with 32.43 % while Gemlik ranked at the bottom with 11.94 %. As well as the number of perfect flower in 2017 was concerned maximum values were recorded for the varieties of Frantoio (85.34 %), Chietina (84.84 %), Nabali (84.13 %), Azerbaijan (83.56 %) and Gemlik (83.11 %).

However, Ottobratica remained at the bottom with (62.69 %). In 2018 the increasing trend in perfect flower was found in all most all the varieties which ranged from 67.57 % to 88.06 %. The variety Gemlik (88.06 %) was ranked at the top while Ottobratica (67.57 %) remained at the bottom. All other varieties ranged in between these two extremes.



Fig. 3. A view of pollen viability under electric microscope of different olive varieties.

Many other studies reported that the percentage of staminate to pistilate flower or perfect flower may vary with the genotype, environmental condition, shoot to shoot and from off to one year production (Fabbri *et al.*, 2004; Martin and Sibbett, 2005). We have found more number of perfect flowers as compared to staminate flowers in all most all the varieties which are in accordance with the other findings. The reports described that perfect flower percentage ranged from 20 to 96 (Cuevas *et al.*, 1994;

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Dimassi *et al.*, 1999). So, it is concluded that overall production of flower in an area is dependent upon environmental conditions, balance nutrition and hormone, cultural practices and other factors.

#### Fruit set percentage

The data for final fruits et was recorded after one month of initial fruit set. The statistical significant variation was found among the varieties and between the years. In 2017, maximum final fruits et was found in variety Moraiolo (4.23 %) followed by Coratina (3.69 %) and Nabali (3.65 %). The minimum values for final fruits et percentage was recorded in variety Earlik (1.38 %) nearby Nocellera (1.48 %), Leccino (1.67 %), Correggiolo (1.69 %) and Ottobratica (1.75 %) as shown in Table 3. In 2018, an increasing trend for fruits et percentage was recorded in almost all the varieties. However, varieties did not follow the same pattern against ranking on the basis of final fruits et percentage. Variety Coratina snatched the top position with 3.75 % from the variety Moraiolo who fell down at six positions (3.22 %) in comparison of 2017. The variety Nocellera ranked at the bottom position with 1.77 % final fruits et percentage with nearby varieties of Ottobratica (1.87 %) and Leccino (1.97 %). All other varieties ranked in mediocre position with 2 % to 3.5 %.



Fig. 4. Olive varietal response towards pollen germination (%) during 2017 and 2018.

It is believed that fruit set of 1 % to 2 % is considered to be a commercial yield in a year of ideal flowering (Fabbri *et al.*, 2004; Martin and Sibbett, 2005). Fruit set is more (up to 10 %) in off year when there is weak flowering and less fruits et in the year of heavy flowering during on year (Martins *et al.*, 2006) and our findings were similar to above mentioned reports. Fruit set per inflorescence vary with the genotype especially small size or bold size fruit. If one fruit per inflorescence is harvested it will be good except some small size fruit varieties like Arbequina and Koroneiki (Connor and Fereres, 2005). There might be many other reason like flower quality, pollen viability, pistil abortion and nutritional imbalances along with the effect of genotype and locations. Some reports suggested that successful fertilization and fruitlet persistence is directly dependent upon flower quality (Martins *et al.*, 2006; Rapoport and Martins, 2006) which also predict pistil abortion rate, receptivity of stigma and pollen viability (Moreno-Alías *et al.*, 2018). In the present study, it was noted that fruits et differ from first year to second year which might be due to the quality of flower which was better in off year than on year production. These findings are similar to the findings of Mazzeo *et al.*, (2014) who said that flower quality vary with the off and on year production and large size fruit varieties have maximum fruits et percentage which may be associated with large ovary or pollen size. The results were in agreed with the findings of Cuevas and Polito, 2004 who reported that large ovaries are associated with more fruit set.

## Pollen viability and germination

The viability of the pollen grains was determined over a period of 2-years (Fig. 3). Floral shoots were randomly selected before anthesis for each cultivar. It is believed that pollen ability is an important tool for pollination process for mono orchard cultivation. The glance of the Fig. 2 depicted that there was statistically significant variation among varieties was found regarding pollen viability for both the years. In 2017, the pollen viability was found maximum in variety Gemlik (53.07 %) closely followed by Frantoio (52.46 %), Ottobratica (49.55 %), Manzanilla (49.46 %) and Nabali (48.11 %). The variety Leccino depicted poor performance regarding viability of its pollen with (27.16 %). All other varieties remained in between 32 to 43 % range. As for as pollen viability for 2018 was concerned the results were found statistically significant. Variety Gemlik remained again at the top position (79.13 %) closely followed by Nabali (77.15 %), Ottobratica (76.52 %), Manzanilla (74.33 %), Moraiolo (74.29 %) and Chietina (72.81 %). The variety Leccino remained at the bottom with (47.62 %) pollen viability. From the Fig 2, it is concluded that varieties Azerbaijan, FS-17, Pendolino, Leccino, Earlik and Sevillano fell in the same group ranges from 57 to 60 % pollen viability. In comparison of both the years, it could be concluded that pollen viability is highly depended upon number of pollen produced, genotypes, high temperature and last year production. As it is evident that in 2018, pollen viability was more as compared to 2017.

As for as pollen germination was concerned the variation was found statistically significant in both the years (Fig. 4). In general, it was observed that varieties which have more viable pollen have more

germination capability. In 2017, the variety Ottobratica (33.66 %) remained at the top followed by Gemlik (30.55 %), Frantoio (29.89 %), Manzanilla (29.72 %) and Pendolino (27.82 %) in pollen germination capability. As usual Leccino remained at the bottom with 12.75 % germination nearby varieties Correggiolo (13.95 %) and Chietina (14.16 %). All others varieties remained in between the values of 14 % to 23 %. In 2018, Ottobratica again showed maximum germination 58.46 % closely followed by Gemlik (58.44 %) and Pendolino (51.15 %). The varieties Leccino (25.74 %) and Correggiolo (26.24 %) ranked at the bottom level. Varieties Chietina, Coratina, Nabali, Moraiolo, Hamdi, Frantoio, Manzanilla, Nocellera, Azerbaijan and sevillano categorized in same group with the range of 32 % to 48 % pollen germination. The remaining three varieties viz-a-viz Fs-17, Souri and Earlik have almost same pollen germination capacity ranged with 28.02 % to 28.89 %. From the Fig. 4, it is concluded that more pollen germination percentage was found in 2018 as compared to 2017.

In the present study, we found a strong variation in pollen viability and germination capacity between cultivars under study. Overall results indicated that varieties showed poor performance regarding said parameters in comparison to olive growing in Mediterranean climatic condition might be due to high temperature, dry winds and poor conditions of soil. As a result poor fruit set was achieved. In other studies it was well reported that poor pollen viability and germination percentage caused less fecundation in olive (Bini, 1984; Tombesi, 2013). No doubt the trait of pollen viability is strongly associated with the genotype (VuletinSelak et al., 2014). We concluded that pollen viability was less as compared to ideal climatic conditions of Mediterranean region which showed dissimilarities to the finding of Pinillos and Cuevas, (2009) who said that cross pollination is not a limiting factor for commercial yield in multivariate block. However, it is well clear that for commercial level of fruit set olive need a huge quantity of viable pollen (VuletinSelak et al., 2014; VuletinSelak et al., 2011).

### Conclusion

In the process of evaluating the performance of exotic olive genotypes, it could be concluded that olive phenology and pollen parameters are not only genotypic characteristics but there are also other a biotic and biotic factor specially temperature has prominent role. All the varieties in each parameter showed variation but over all varieties Gemlik, Coratina, Frantoio, Nabali, Hamdi, Moraiolo, Chietina could be recommended for olive orchard establishment. The variety Ottobratica could be used as pollinator on the basis of good pollen viability and germination capacity.

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