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ORIGINAL ARTICLE

TEST OF A STUDY OF PHENOLOGY OF PISTACHIO FRUIT (*PISTACIA VERA* L.) IN THE ORCHARD TIGHENNIF (W. MASCARA, ALGERIA)

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ABSTRACT: Among the cultivated species, hardy and resistant to harsh climatic and soil conditions, the true pistachio (*Pistacia Vera* L.) is one species that has unique characteristics (plasticity, hardiness, high tolerance to climatic variation, adaptation to different soil types etc.), which allows it to be cultivated in arid and semi-arid. Species of the genus *Pistacia* are dioecious and Wind-pollinated with alternating in fruiting. Pistachio varieties are usually self-sterile. The reasons for this sterility are many: shifting maturity of male and female organs, there are separate male and female flowers and especially hormonal or genetic incompatibility. This allows the mixing genetic incompatibility for the survival of species in nature. The annual growth cycle of tree species in temperate climates can be summarized in a phase of winter dormancy followed, or preceded by a period of summer growth. In the part of materials and methods, we were interested in fruit pistachio (*Pistacia Vera* L.) to study the phenological behavior. The five female varieties are: Achouri, Batouri, Neb. djamel, and Bayadhi Adjmi. Pollinators are summarized: Male Khalifa Jaber Male and Male Jamil. As such, during this work, we set ourselves two objectives:
- The phenology of the species, to define the developmental stages of the growth cycle;
- The qualitative and quantitative study of pollen.

KEYWORDS: Pistachio (*Pistacia Vera* L.), Growth, Dormancy, Bud Phenology, Growth Cycle, Male and Female Inflorescence.

INTRODUCTION

Phenology can be defined as "the study of the temporal distribution of recurring biological phenomena, the causes of their timing in relation to biotic and abiotic forces, and inter-relationships between the phases within the same species or between different species" (Ghrab et *al*., 2009). These periodic phenomena distinguish states that are the phenological stages of the life cycle of an organism. These cycles occur yearly in both the animal kingdom (migration, hatching, raising diapause etc.), as in plants (buds, flowers etc.).

Among the cultivated species, hardy and resistant to harsh climatic and soil conditions, the true pistachio (*Pistacia Vera* L.) is one species that has unique characteristics (plasticity, hardiness, high tolerance to climatic variation, adaptation to different soil types etc.), which allows it to be cultivated in arid and semi-arid. Species of the genus *Pistacia* are dioecious and Wind-pollinated with alternating in fruiting. Pistachio varieties are usually self-sterile. The reasons for this sterility are many: shifting maturity of male and female organs, there are separate male and female flowers and especially hormonal or genetic incompatibility. This allows the mixing genetic incompatibility for the survival of species in the wild (Afshari et *al*., 2008). It also remains that even in many situations of self fertility, the contribution of external pollen (cross fertilization) results in a faster fruit set, fruit development better and better resistance to frost (Atli et *al*., 2007). Pollination is the transport operation of pollen on the pistils. It is done by insects (entomophilous) especially by bees, hence their importance to the grower, it is also done by the wind (anémogamie) and water (hydrogamie). As such, during this work, we set ourselves two objectives:

- The phenology of the species, to define the developmental stages of the growth cycle;
- The qualitative and quantitative study of pollen.

MATERIALS AND METHODS

2.1. Study Area

This work was conducted at the experimental farm, demonstration, (TTAF) of Tighennif (W. Mascara). This farm of 53.00 acres divided into UAA 08.00 hectares of vineyard planting and 4.00 ha of pistachio orchard fruit, the rest is bare earth. The station is 20 km from the capital of the wilaya of Mascara (Algeria). The study area is an orchard of fruit Pistachio of 04 hectares

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where the planting was done based on several Syrian varieties planted between 1981 and 1986 as part of cooperation between ACSAD and ITAF, this orchard is designed to the production of scions for propagation and spread of clones of other lumberyards.

2.2. Organic Materials

In this part of our work, we were interested in pistachio fruit (Pistacia *V*era L.) to study the phenological behavior. The five female varieties are: *Achouri, Batouri*, *Neb djamel*, *Bayadhi* and *Adjmi*. Pollinators are summarized: *Male Khalifa, Male Jaber* and *Male Jamil*. Male genotypes to plant must comply with a sex ratio of 1/15 to 1/8 for a good or an anemophily entomophily. Male trees are scattered throughout the orchard with a localization of the prevailing wind side, if the land is windswept. Nevertheless, it would suffice to plant some trees 12p.100 pollinators, or about 1 foot male to female 8 feet or 1 foot to 15 foot male female.

2.3. Method of Work

Based on the detailed map of the orchard of pistachio fruit set in June 2006 by the research team's current project, in collaboration with engineers from the demonstration farm Tighennif. We opted for 05 female varieties (Achouri, Batouri, Neb djamel, Adjmi and Bayadhi), including 07 individuals each, with considerable economic interest, and 03 pollinators males (Male Khalifa Jaber Male and Male Jamil) in which individuals are selected with reference to certain criteria such as (the diameter, the port, and an interesting phenotypic value) for phenological monitoring. For each individual, four branches corresponding to exposures North, East, South and West were surrounded by a brightly colored ribbon and were followed for the duration of the study. Of temperature measurements were made once a week. For this purpose, a thermometer was placed on a branch of a tree plot that has maintained throughout the observation period. Our observations began on 12/02/2008 and ended on 18/09/2008.

2.4. Qualitative and Quantitative Study of Pollen

Research on the improvement program agronomic species interests have grown considerably in recent years, they require fast and accurate techniques to characterize the pollen and to determine in particular stages of pollen development, both on its quality of its viability. We chose three male pollinators of pistachio fruit, we waited for the flowering of each pollinator then we took a floral bouquet at the beginning of flowering. We took aluminum foil and a beaker of water and put the bouquet in it (see Figure 1). After isolating the dried flower parts are recovered gains of pollen and pure dry powder form, they are kept in sealed vials at a temperature of 4 ° C. To characterize the quality of pollen produced by 03 male pollinators, we proceeded in two steps:

Step 1: Test of pollen viability

Pollen and harvested which have a phenological monitoring at Jaber Male, Male and Male Jamil Khalifa were tested at the stage of dehiscence of the stamens (pollen availability) by the method of Acétocarmin (90 cm 3 of acetic acid, we add 110 cm3 of distilled water, heated to boiling, we add a few drops of carmine and one to two drops of iron as a fixative) (Abu-Zahra and Al-Abbadi, 2007). On a slide, you put a small amount of pollen to which is added a drop of Acétocarmin. Counts and observations are carried out under light microscope (Gr 100x40) and (Gr 100x100). The fertile pollen grains appear colored in red and non-viable are not colored (transparent).

Figure 1: The male floral bouquet and the recovery process of pollen grains

Step 2: Test of pollen germination

We collected pollen on 17/04/2008, This coincides with dehiscence of the male flowers of the majority of clones.

To test the germination of pollen from male plants, we used two different culture media compounds (Table 1).

Table 1: Composition of culture media for germination

Culture medium A	0.5g/l borique acid + 10 % de sucrose	
Culture medium B (Milieu de Bewbaker et Kwack modifié)	H_3BO_4	500 mg
	$Ca(NO_3)_2.4H_2O$ $Mg SO_4$	300 mg
	$Mg SO_4$	200 mg
	KNO_3	100 mg
	Na_2Mn	100 mg
	Saccharose	100g
	Eau	1000 ml

The culture media thus prepared were autoclaved at 180 ° C for 20 min.
This culture medium was transferred to sterile petri dishes where we cultured pollen. Observations and counting of germinated pollen were made under light microscope at magnification (Gr 100x100).
The evaluation of pollen germination rate was made after 24 pm and 48 pm on 18/04/2008 and 19/04/2008

RESULTS AND DISCUSSION

3.1. Phenology

It is recognized that in dioecious fruit trees such as pistachio, parthenocarpic fruit production results from a phase shift between the two phenological features male and female (Acar *et al.*, 2006). As the first objective we set ourselves was to study the flowering times of male and female individuals of Pistachio, we characterized the different phenological stages that coincide with the dates of appearance of different phenophases such as bud break, flowering and fruiting.

3.2. Determination of the Different Phenological Stages

3.2.1. Budding Vegetative

In our study, the duration of bud break is longer in males, which may take up to two months (February and March), by cons in female individuals is less important, it varies between 20 to 40 days (from March 10 to April 20).
Our results are consistent with those of Timothy *et al.*, (2007) which characterize the phenology of pistachio true in Syria, said that the dormancy period is longer in male clones, this is consistent with the fact that the female varieties have a dormancy faster and a period of earlier flowering, which explains why the female plants before debourment of male clones. We could identify, in regard to the true pistachio (Pistacia vera L.), various stages of budding which are distinguished by three life stages of the growing season:

Stage 1: Swelling of buds: (S0-S1)

We observed that this phase coincides with an increase in maximum temperatures of 10 ° C. In early February, the vegetative buds begin to swell, either for males or female individuals; this is explained by the formation of new cells related to cambial activity (Afshari et al., 2008).

Stage 2: Budding buds: (S2-S3)

We noted that vegetative bud occurs at different times, for males and females. Indeed, from the end of March, the vegetative buds of female plants begin to clogs. By cons in males vegetative bud break occurs after flowering of the latter. It should be noted that vegetative bud is characterized by the breakup and the opening of buds, which correspond to the scales open and their spacing, thus letting new growth of a compact light green.

Stage 3: Elongation of buds (S4)

At this stage and from the beginning of the month of April, the first leaflets appear and grow until they reach their maximum size, which varies from one variety to another. Indeed, we observed that leaf length varieties females were greater than those of males. This phase starts from 07/04/2008 to 14/04/2008, for clones precocious males; duration does not exceed 08 days.

3.2.2 Flowering

3.2.2.1. Flowering of male feet

For our test, flowering fruit of the pistachio started from the end of March 2008. For individuals' precocious males (Male Jaber), flowering began March 27, 2008, and this after flowering bud breaks. As for intermediate male clones, the case of male Jamil, flowering varies between 02 and 14 April 2008. While the late clones, such as Male Khalifa, flowering started from April 17, 2008. During this phase, we identified four phenological stages.

Stage 1: Inflorescence pooled (S1)

During this stage, the male flowers have a bright red color and are inserted one another, forming a compact mass (Figure 2).

Stage 2: Dehiscence of inflorescences (S2)

At this stage, the male flowers have a less intense color and are less compact. Moreover, it has an elongation of the axis which bears the flowers. These become less frequent, their color becomes yellowish red (Figure 2).

Stage 3: Dehiscence of inflorescences (S3)

This phase began in 2008 at the 07Avril April 14, 2008. For male clones early, its duration does not exceed eight days. As for the male intermediate phase began dehiscence of 17/04/2008 to 24/04/2008. For males the late phase of dehiscence begins on 24/04/2008 to 30/04/2008, the duration of pollen availability does not exceed seven days. Inflorescences reach their maximum size (2 to 3 cm long), color turns yellow and the axis which bears the flowers growing (Figure 2).

Stage 4: wilting of inflorescences (S4)

After pollination, we observed drying, followed by a blackening which led to the downfall of the inflorescences. This stage also corresponds to the recovery of activity in vegetative buds, observed in clones of male pistachio tree; these will produce new leaves for next year (Figure 2).

Figure 2: The different stages of flowering male fruit of the pistachio (*Pistacia Vera* L.)

3.2.2.2. Flowering of female feet

For monitoring phenological female flowers, we took into account the aspect of stigma and we distinguished four stages of development of female flowers as follows:

Stage 1: No Apparent Flowers: (S_0-S_1)

During this stage, the blanks are visible, we see against the female flowers are invisible because they are covered with scales. This does not yet allow us to distinguish the stigma (Figure 3).

Stage 2: Early onset of flowers (S_2)

We note the appearance of female inflorescences, caused by the bursting of shells, which partially reveal small flowers 3 to 4 mm long, yellow. At this stage the stigma becomes apparent (Figure 3).

Stage 3: Extension of inflorescences (S_3)

We note at this level, the color becomes light pink flowers, which characteristic of female receptivity (this is the stage where the stigma may be receptive). The duration of receptivity is 08 days for the majority of individuals studied; inflorescences become more elongated and can reach 8 to 12 mm (Figure 3).

Stage 4: Flowers pollinated (S_4)

We notice a color change of the stigma that goes black, which tells us that at this stage the flowers are pollinated. The stigma is more likely to receive pollen (Figure 3).

3.2.3 Fruit

At this stage we note the appearance of the fruit in the form of small bulbs, which will grow into mature fruits (Figure 3). According to Kuru and Aksu, (2005) growth in length and diameter of the fruit of the pistachio true after fertilization, show three distinct phases: two cycles of rapid growth separated by a very slow growth cycle. Indeed, our observations we have identified three phases of growth:

First Phase

This is a phase of rapid growth of the pericarp, which runs from early April until mid-May, in which the pericarp reached almost the maximum size that varies between 0.2 to 0.8 cm lengths according to the three varieties studied. As for the weight of the fresh almond, it can reach 0.5 g, representing about 80% of fruit weight.

Second Phase

We observe that during this phase, which lasts from mid-May until late July, the period of growth is very slow and hence the increase in size of the pericarp is insignificant. According to Marra *et al.*, (2005), a month after this period, the developing embryo becomes visible; the kernel begins to grow with the rapid accumulation of sugars and their conversion into fat. This is the end of this period that the kernel is almost its final size of 2.5 to 3 cm (early July), and that happens hardening of the endocarp.

Third Phase

With respect, it takes about six weeks (from late July until September) According to Ozker *et al.*, (2006), this period corresponds to an increase in total wet weight as a result of the magnification of the kernel. Indeed, we estimated the weight of the kernel, the different varieties studied, which can reach 2 g fresh weight of fruit in the variety Achouri, 1.7 g for the variety Batouri and 1.2g for the variety Oleimi. At this stage, there is a slight increase in the size of the pericarp and the

endocarp dehiscence. The conversion of sugars into fat ends at the end of September, the fruit became ripe and edible Ozker et *al*., (2006).

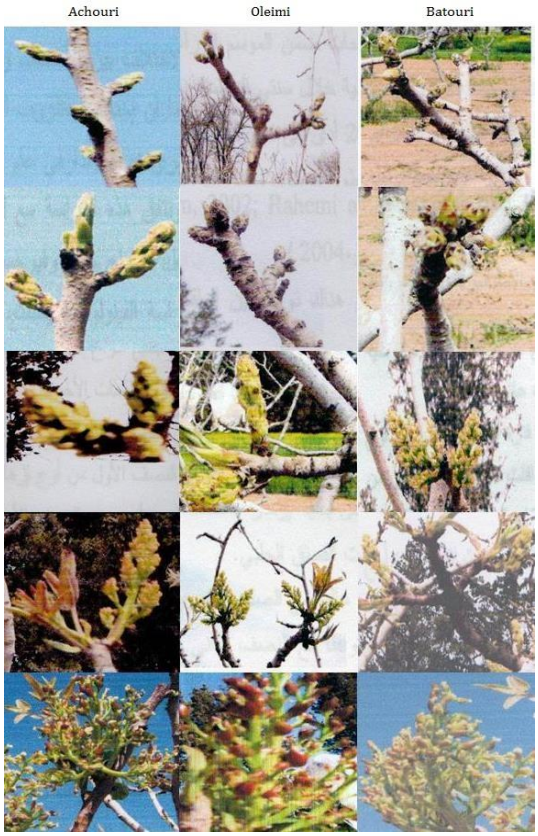

Figure 3: Different stages of female flowering of some varieties of pistachio.

From Table 2, we notice a change in varieties of grapes pistachio fruit. The rate of cluster ranges from 54.82% (Male Khalifa) to 70.1% (Male Jamil), which verifies the effects of pollinators on the number of clusters per variety. While, the average cluster varies by varieties of 55.6% (Neb djamel) and 77.73% (Bayadhi). The figure 4 reveals the effect of pollinators on the number of bunches of grapes among different varieties studied.

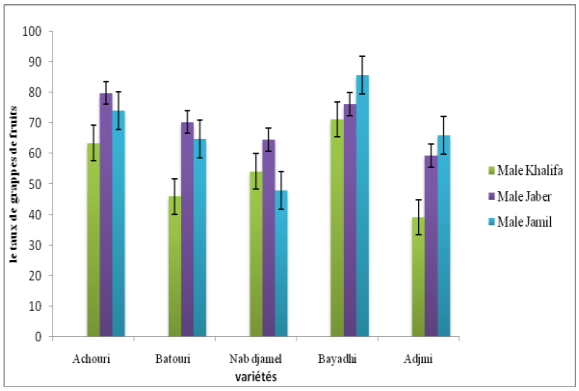

Figure 4: effect of pollinators on the number of bunches of grapes in different varieties studied.

χ^2 test was used to show this difference and a calculation of χ^2 (χ^2 = 15.8) using the following formula:

$$\chi^2=\sum(o_i-c_i)^2/\ c_i$$

Where,
o_i: observed numbers
c_i: calculated numbers obtained by the following rule: this = row total * column total / total. The table 3 is a contingency table.

Table 2: Effect of pollinators on the rate of fruit clusters for the varieties studied

Variety / Pollinators	Achouri	Batouri	Nab djamel	Bayadhi	Adjmi	average
Male Khalifa	63.5	46.0	54.2	71.2	39.2	54.82
Male Jaber	79.9	70.4	64.6	76.2	59.4	70.1
Male Jamil	74	64.9	48	85.8	66.1	67.76
average	72.46	60.43	55.6	77.73	54.9	
LSD	females		Pollinators		females × pollinators	
0.05	9.45		15.43		26.73	

Table 3: Contingency table including varieties and pollinators

Variety / Pollinisators	Achouri	Batouri	Nab djamel	Bayadhi	Adjmi	Total lines
Male Khalifa	63.5	46.0	54.2	71.2	39.2	274.1
Male Jaber	79.9	70.4	64.6	76.2	59.4	350.5
Male Jamil	74	64.9	48	85.8	66.1	338.8
Total columns	217.4	181.3	166.8	233.2	164.7	963.4

Comparison with χ^2 (α = 5%) = 18.3070 (Law Chi-square) for df 10 (5-1) (3-1), explains the difference.

3.3. Qualitative and Quantitative Study of Pollen

The objective of this study was to test the quality of pollen produced by male clones studied to select the best, in view of integrating them into a breeding program heads of clones in the future.

3.3.1. Test of Pollen Viability

According to the chart below and after counting under a microscope (x100 GR100), we noticed that the majority of pollen grains are stained red (from 70% to 100%). Indeed, male clones Jamil Khalifa and the rate of color have the highest (100%) (Figure 5, a). Against by the lowest rate is found among the male clone Jaber with 50% staining. However, we point out that the rate of stained pollen grains is ten times the number of pollen grains unstained.

Table 4: Rates of pollen stainability by the solution of acetocarmine

Male Clones	% Cloning Rate
Male Jamil	75
Male Khalifa	95
Male Jaber	50

The red color of the pollen grains is a good indicator of the viability and effectiveness of pollen clones tested. According to Izzat and Sinan, (2007) pollen grains are stained red and turgid have a homogeneous cytoplasm. However, according to the same author, this staining is overestimating the fertility of pollen grains.

3.4. B-Pollen Germination Rate Based On Male Clones

Pollen germination and pollen tube growth were studied to test the compatibility of one or more cultivars, in order to discover the causes of anomalies or accidents related to pollination and fertilization. Thus we studied the germination of pollen grains of male clones of the pistachio true. Counting under a microscope cover all of the pollen grains, found in the culture medium, for each clone tested (Figure 5, b). The test results of pollen germination of these clones that were used for testing are listed in the table 5.

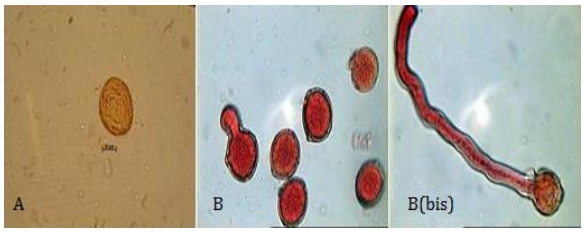

Figure 5: Determination of pollen viability in the pistachio fruit. a: Viable pollen (male Khalifa) (Gr100*100), b: Beginning of germination of a pollen grain, b (bis): Germination after 6 hours.

Table 5: Evolution of the percentage of pollen germination versus time

Males	Germination after 24h of culture (%)	Germination after 48h of culture (%)
Male Jaber	25	30
Male Khalifa	23	38
Male Jamil	20	36

From Table 5, the percentage of pollen germination after 24 h of germination is below the average compared to rates obtained by Rahemi and Abdollahi, (2004). In addition, pollen grains still germinate even after 48 hours, this would be that the germination of pollen tubes do not stop until the pollen grains are found in this culture medium. According to Rahemi and Abdollahi, (2004), pollen germination on artificial medium, shows that the rate of grain sprouts and length of pollen tube vary depending on the sucrose content.

The optimum concentration of sugar is 20 to 30%. Germination begins to decrease when the concentration exceeds 40% sucrose. Our results on a culture medium to 20% sugar, we found that the percentage of germination after 24 hours, between 20% (Jamil Male) and 25% (Male Jaber). After 48 h of cultivation, germination reached 36% (Jamil Male) and 38% (Male Khalifa). Therefore, it seems likely that the concentration of 20% sucrose gives interesting results, an average of more than 31% germination of the pollen tube for most of the male clones studied. These rates reflect an average viability and pollen moderate efficacy of these clones. Indeed according to Rahemi and Abdollahi, (2004), pollen grains with good viability and high efficiency should have a rate exceeding 50% germination.

We noted that in our experiment, we observed in the month of April 1 attack of an insect beetle (lady bug) infesting leaves, and also in the month of June until September, we also observed another supposed fungal attack but could not determine the species. This would be a fungus *Alternaria sp*.

CONCLUSION

Phenology of pistachio fruit is proportional to the date of bud that is a key character for this vital activity of plants. It is part of the annual cycle of alternating phases of growth and dormancy, which provides a synchrony to the tree screw-vis Environmental conditions, including the winter phase is highly unfavorable to growth. The character is both under the influence of biotic factors (populations of defoliating insects) and abiotic (weather) and can be modeled as a first approach with the temperature. But beyond the predictive models,

the need is great to dissect the molecular mechanisms controlling the phenomenon of bud break to better understand the effect of climate on character. Values exhibit a bud-like continuous phenotypic variation normal distribution characteristic of a quantitative character says, or complex, placed under the control of many genes and environmental factors. The phenotypic value (P) can be decomposed into a genotypic value (G), under the control of a number of genes, and environmental value (E) induced by environmental factors. If we neglect the effects of interaction between components, the equation P = G + E summarizes this principle.

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