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# Flower description and fertility of the main Tunisian almond cultivars

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**Abstract.** The almond *Prunus dulcis* (Mill.) D.A. Webb is an old edible nut that is present in Tunisia with a large phenotypic and genetic diversity of cultivars and landraces. However, no much investigation has treated the characterization of the local cultivars regarding flower description and fertility parameters. This study is dealing with the evaluation of the almond genetic diversity on the basis of some parameters related to flower characteristics. Also, the quality of pollen as well as the fructification rates for self and cross-pollination were studied in order to differentiate among genotypes according to their degree of self compatibility or incompatibility. Results showed a great intraspecific genetic diversity. Analysis and observations allowed us to distinguish some particular genotypes such as 'Zahaf' which is characterized by its largest flower size. Also it was concluded that 'Achaak 2' has the highest potential of fertility (80%) and a considerable fruit bearing and productive capacities. 'Mahsouna' and 'Tlili 8' presented the highest rates of fruit set when self-pollinated and as a consequence showed a tendency for self-compatibility.

**Keywords.** *Prunus dulcis* L. – Flower descriptors – Pollen fertility – Self pollination.

***Description florale et niveau de fertilité des principales variétés tunisienne d'amandier***

***Résumé.*** *L'amandier* Prunus dulcis *(Mill.) D.A. Webb est une espèce fruitière de culture très ancienne en Tunisie. Elle compte de nombreux variétés et écotypes ayant une diversité phénotypique et génétique importante. Toutefois, peu de travaux se sont intéressés à la caractérisation de cette richesse en se référant aux caractéristiques morphologiques de la fleur en relation avec la biologie de fructification. Cette étude se propose la caractérisation de la diversité génétique locale d'amandier en se basant sur de nouveaux descripteurs morphologiques pour les différentes pièces florales. Aussi, on s'est intéressé à l'évaluation de la qualité du pollen et des niveaux d'auto-fertilité et/ou d'autostérilité des principaux génotypes d'amandier. Les résultats sont très encourageants et nous ont permis de prouver d'une part la richesse de notre patrimoine génétique en amandier ainsi que sa diversité et de faire ressortir d'autre part certaines spécificités variétales. D'ailleurs, les deux variétés 'Zahaf' et 'Achaak' se sont bien distinguées, la première par la taille de la fleur et la deuxième par le niveau de fertilité élevé et par conséquent des potentialités de fructification assez remarquables. Finalement, il s'est avéré que les variétés 'Mahsouna', 'Porto' et les écotypes 'Tlili 8', 'Amara4' présentent des taux d'auto-pollinisation satisfaisant et donc des tendances vers l'auto-compatibilité.*

***Mots-clés.*** Prunus dulcis *L. – Fertilité pollinique – Descripteurs floraux – Autopollinisation.*

## I – Introduction

Almond [*Prunus dulcis* (Mill.) D.A. Webb] belongs to the genus *Prunus* and the large family of the Rosaceae. The world production is estimated to 2.00 million tons per year (FAO, 2011). For this species flowering, flower quality and fruit set are of great economical importance while yields depend on them. Moreover, it is largely self-incompatible and necessitates cross-pollination, with overlapping bloom time to ensure good yield (Sharafi *et al.*, 2010). Nevertheless, few studies were dealing with flower biology for almond. The almond descriptors established by the former International Board for Plant Genetic Resources and edited by Gülcan (1985) present only very few parameters such as self compatibility, colour of petals and number of pistils.

The aims of this study are to present new almond descriptors related to flower components, to demonstrate the large diversity existent in the local germplasm expressed by flower descriptors and to estimate the level of self compatibility for the main local cultivars.

## II – Materials and methods

Twenty-eight local genotypes and four European cultivars were characterised using 14 descriptors related to flower anatomy. They were preserved in the national collection of Sidi Bouzid (35.117 N, 9.567 E; 369 m above sea level.). Trees were drip irrigated grafted on 'Garnem' rootstock and receive conventional technical practices. Descriptors used in this study were related to petals (colour, number, length and width), stamens (number, length of the filament, colour of the filament), pistils (number, length and colour), calyx diameter, the position of the pistil regarding the stamens, the colour of the attachment point of petals and to the ratio length/width of petals.

The pollen viability tests were done individually. Acetic carmine was used for pollen coloration. Viable pollens were dyed in red and light red; dead pollens were not dyed. The pollen rate viability (PRV %) was calculated as the ratio PVR % = (number of dyed pollen / total pollen number)*100.

Pollen germination rates were analyzed on agar sucrose based media described by Hedhly *et al*. (2004) an in Petri dishes at 20°C according to Pinney and Polito, (1990). Observations were done after 2, 4, 6 and 24 hours and the germination was stopped by adding formaldehyde to the Petri dish. Observations were done using a light microscope (Leica, Germany) with 10 x ocular and 40 x objectives.

For self pollination trial three branches per tree were chosen and the number of flowering buds was registered. Self-compatibility levels were estimated by the number of fruit set. For that three branches/tree were covered before blooming by insect-proof cheesecloth cages. For each branch the number of flowers was noted. After petal fall the bags were removed. Data presented in this paper reported two dates of fruit sets. The first after removing the bags (D1) and the second (D2) two months after the end of physical fruit drop in almond (Kester and Griggs, 1959).

For open pollination three branches/tree were marked, flower buds and fruit set consequently were recorded. Fruit set was calculated for different pollination treatments and dates by dividing the number of fruit set by total number of flowers present on the shoot.

For statistical analysis an ANOVA was done using SPSS 17.0 and the differences between means were compared using Duncan test at $p \leq 0.05$.

## III – Results and discussion

The principal component analysis (PCA) realized for the 33 genotypes with the 14 flower descriptors showed that the two first principal components explained 42% of the total variability observed (Fig. 1). The first axis was formed by petal length (PL), petal width (PW), pistil length (PSL) and calyx diameter (CD). The second axis was associated with the petal colour (PC), the stamen filament colour (FC) and the colour of the point of petal attachment (CAPP). Consequently, it is assumed that the local cultivar 'Zahaaf' was distinguished by the large size of its flower. In the opposite side it was demonstrated that even though the cultivar 'Achaak' is genetically distinct from 'Mazetto' they seemed to have close flower sizes. In the upper left quarter part of Fig. 1 is clearly noticeable the ecotype 'Amara 4' presenting special colours for petals, stamen filament and petal attachment point.

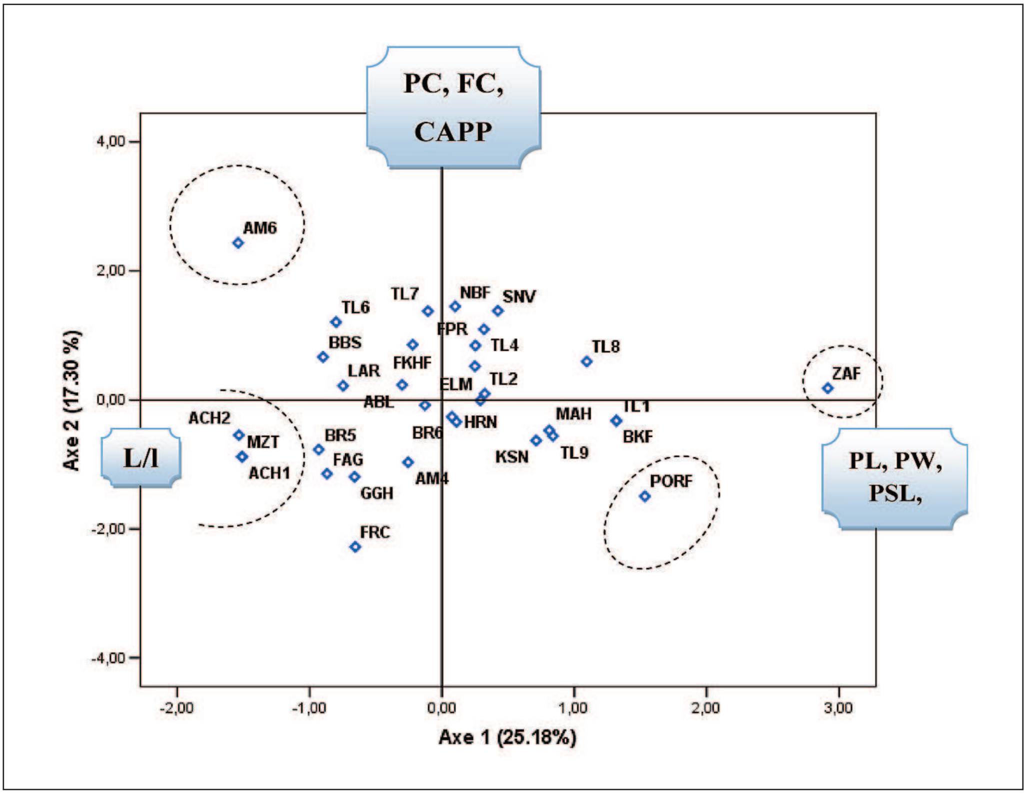


**Fig. 1. Principal component analysis for 33 almond genotypes based on 14 flower descriptors.**

Pollen viability test was very expressive while all the self-compatible cultivars presented values higher than 60% (Table 1). For the local genotypes values ranged from 37.5% for 'RasBouma' to 79.9% for 'Tlili8'. High values were also observed for 'Porto' (76.2%), 'Achaak' (72.1%), 'Amara 4' (67.9%), 'Elloumi' (62.4%). The high fertility of the ecotype 'Tlili 8' was also expressed by the germination rates presented after 2, 4, 6 and 24 hours (Table 1). In fact 57.6% of the pollen germinates after only two hours. Similar value (53.9%) was reached for 'Porto' after four hours and for 'Lauranne' after 24 hours. At this last date a clear superiority was observed for many local genotypes in comparison to the introduced ones. That was clear for 'Amara4' (57.5%), 'Porto' (70.6%) and 'Elloumi' (56.9%). This may be due to the fact that they need a relatively high temperature similar to that prevails during the month of March when these cultivars bloom. Differences in pollen performances have been also noted, in different genotypes of almond (Martínez-Gómez *et al*., 2002) and apricot (Egea *et al*., 1992).

Results regarding self pollination treatment for the 10 local almond genotypes and the 4 introduced cultivars revealed that fruit set rate means after petal fall were highly variable for all locals (Table 2). In fact, they varied from 0% for 'Achaak' to 37.6% for 'Mahsouna'. This confirms previous annotations that the more famous Tunisian almond cultivar 'Achaak' is strictly self-incompatible. High values were noted for 'Tlili8' (31.3%), 'Porto' (28.5%), 'Bouchouka KF.' (18.7%) and 'Tlili9' (17.3%). Nevertheless, for the open pollination treatment and at the same date fruit set values were even higher. They varied from 26.4% for 'Achaak' to 68.3% for 'Bouchouka KF'.

**Table 1. Pollen viability (VR%) and germination rates after 2, 4, 6 and 24 hours for some local and introduced almond genotypes**

| | Cultivars | VR (%) | GR 2 h (%) | GR 4 h (%) | GR 6 h (%) | GR 24 h (%) |
|---|---|---|---|---|---|---|
| Local Cultivars | 'Achaak' | 72.1 | 3.7 | 6.6 | 3.7 | 39.2 |
| | 'Amara4' | 67.9 | 17.7 | 17.4 | 24.5 | 57.5 |
| | 'Bouchouka B.S.' | 48.4 | 9.2 | 9.1 | 17.5 | 28.9 |
| | 'Bouchouka K.F.' | 37.7 | 17.3 | 25 | 34 | 31.5 |
| | 'Elloumi' | 62.4 | 7.8 | 35.6 | 40.7 | 56.9 |
| | 'Mahsouna' | 56.3 | 7 | 6.3 | 16.8 | 20.7 |
| | 'Porto F.' | 76.2 | 34 | 53.9 | 71.5 | 70.6 |
| | 'RasBouma' | 37.6 | 14.6 | 20.1 | 27.8 | 30.9 |
| | 'Tlili8' | 79.9 | 57.6 | 63.8 | 63.3 | 72.7 |
| | 'Tlili9' | 51.2 | 2 | 8.1 | 9.1 | 42.5 |
| Introduced Cultivars | 'Francoli' | 66.8 | 6 | 12.6 | 18.6 | 40.8 |
| | 'Lauranne' | 71.5 | 30.1 | 15.6 | 20.6 | 57.9 |
| | 'Mazetto' | 74.9 | 17.5 | 14.5 | 19 | 50.6 |
| | 'Supernova' | 58.4 | 14.3 | 17.1 | 15.1 | 45.1 |
| Significance P ≤ 0.05 | ** | ** | ** | ** | ** | ** |

**Table 2. Fruit set of auto and open pollination treatments for some local and introduced almond genotypes**

| | Cultivar | Fruit set % (D1) auto-pollination | Fruit set % (D1) open-pollination | Fruit set % (D2) auto-pollination | Fruit set % (D2) open-pollination |
|---|---|---|---|---|---|
| Local Cultivars | 'Achaak' | 0f | 26.4f | 0b | 9f |
| | 'Amara4' | 16.8cde | 44.8de | 3.4b | 16de |
| | 'Bouchouka BS' | 13de | 64.1ab | 0b | 14.6de |
| | 'Bouchouka K.F' | 18.7e | 68.3a | 0b | 29.7ab |
| | 'Elloumi' | 8.1e | 52.2bcde | 0b | 16.4de |
| | 'Mahsouna' | 37.6c | 49.1cde | 4.4b | 12.1e |
| | 'Porto F.' | 28.5cde | 45.5de | 6.0b | 12.6e |
| | 'Rasbouma' | 6.5e | 65.7ab | 0.6b | 30ab |
| | 'Tlili8' | 31.3cde | 29.2h | 1.6b | 11.2e |
| | 'Tlili9' | 17.3e | 58.2bcde | 0.8b | 18.8cde |
| Introduced Cultivars | 'Francoli' | 69.3a | 64.5 abcd | 17.3a | 23.5abcd |
| | 'Lauranne' | 70.1a | 51.6 bcde | 20.6a | 30.3a |
| | 'Mazetto' | 50.1b | 45.1de | 22.4a | 20.4cde |
| | 'Supernova' | 69.05a | 65.1acde | 17.3a | 17.6cde |

a,b,c,d,e,f,g,h: Signicant difference at Duncan's multiple Range Test (5%).

The tendency for both treatments after two months of fruit sets (D2) was completely different. In fact, compared to D1 fruit set values have sharply decreased for both treatments and for almost all the local genotypes. Except, 'Porto', 'Mahsouna', 'Amara4' and 'Tlili8' presenting respectively 6%, 4.4%, 3.4% and 1.6% of fruit set, all the others were almost null in auto-pollination treatment. This indicates that almost all the local cultivars studied are self-incompatible and therefore need external pollinator with overlapping bloom periods. Since the cultivar 'Achaak' presented the lower value of fruit set for open pollination it is clear the need for this high quality kernel cultivar to select a good pollinator for it. The existence of cross-incompatibility groups in the Tunisian germplasm may be a first hypothesis for the understanding of this result. In fact it is well known that this cultivar is the first to bloom in Tunisia and our results added that it has high fertile pollen. An important increase of the final fruit set values for open pollination versus self pollination was very clear for the local genotypes 'RasBouma' (from 0.6% to 30%) and 'Bouchouka KF.' (from 0% to 29.7%). These values above 25% confirmed potentialities of these cultivars to give good yields.

For the introduced cultivars it was observed that the self pollination has increased fruit set recorded after petals fall but this was not the case at the final fruit set date (Date 2). In fact, for this last date fruit set was even improved for some cultivars by open pollination. Fruit set values increased from 17.3% to 23.5% for 'Francoli', from 20.6% to 30.3% for 'Lauranne' and from 17.3% to 17.6% for 'Supernova'. The few comparative studies of self-pollination and cross-pollination in self-compatible almond carried out with the varieties 'Guara' (Socias i Company and Felipe, 1992) and 'Lauranne' (Legave *et al.*, 1997) encourage the mono-varietal almond orchards. Even though it is true, for self-compatible genotypes we agree with the idea advanced by Dicenta *et al.* (2001) regarding the importance of cross pollination and mixing even for auto-fertile cultivars. Our work finally confirmed previous reports regarding the self-compatibility of the Spanish cultivar 'Francoli' (López *et al.,* 2004).

## IV – Conclusions

The large diversity of the Tunisian gemplasm was clearly elucidated with the new flower descriptors presented in this work. High levels of pollen viability and germination for many ecotypes approve the importance of the prospecting effort and the need to preserve diversity. The dominance of fruit set values for open pollination treatments versus self pollination support the add value of multivariate plantations for both auto-compatible and incompatible genotypes with the necessity of booming overlapping combinations.

## References


**Dicenta F., Ortega E., Canovas J.A. and Egea J., 2001.** Self pollination versus cross pollination of six self compatible almond cultivars: Pollen tube growth and fruit set. In: *Cah. Opt. Mediterr.*, 56, p. 369-372.

**Egea J., Burgos L., Zroa N. and Egea L., 1992.** Influence of temperature on the in vitro germination of pollen of apricot (Prunus armeniaca L*.).* In: *J. Hort. Sci.,* 67, p. 247-250.

**FAO, 2011.** FAOSTAT, 2011. http://faostat.fao.org/.

**Gülcan, R. 1985.** *Almond Descriptors (revised)*. International Board For Plant Genetic Resources. Secretariat, Rome.

**Hedhly Y.A., Hormaza J.I. and Herrero M., 2004.** Effect of temperature on pollen tube kinetics and dynamics in sweet cherry, *Prunus avium* (Rosaceae). In: *Am. J. Bot.,* 91, p. 558-564.

**Kester D.E. and Griggs W.H., 1959.** Fruit setting in the almond: the effect of cross-pollinating various percentages of flowers. In: *Proc. Amer. Soc. Hort. Sci.*, 74, p. 206-213.

**Legave J.M., Richard J.C., Thermoz J.P. and Duval H., 1997.** Lauranne Avijor dans la course. In: *Fruits & Légumes,* 155, p. 36-38.

**López M., Mnejja M., Rovira M., Collins G., Vargas F.J., Arús P. and Batlle I., 2004.** Self-incompatibility genotypes in almond re-evaluated by PCR, stylar ribonucleases, sequencing analysis and controlled pollinations. In: *Theor. Appl. Genet.*, 109, p. 954-964.

**Martinez-Gomez P., Gradziel T.M., Ortega E. and Dicenta F., 2002.** Low temperature storage of almond pollen. In: *HortScience,* 37, p. 691-692.

**Pinney K. and Polito V.S., 1990.** Olive pollen storage and in vitro germination. In: *Acta Hort.,* 286, p. 207-210.

**Sharafi Y., Karimi M. and Ghorbanifar M., 2010.** Study of pollen tube growth, cross compatibility and fruit set in some almond genotypes. In: *Afr. J. Plant Sci.,* 4(5), p. 135-137.

**Socias i Company R. and Felipe A.J. 1992.** Self-compatibility and autogamy in 'Guara' almond. In: *J. Hort. Sci.,* 67, p. 313-317.