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Low fruitfulness in local almond orchards could be due to the inbreeding depression effect

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Abstract

Fruitfulness of 26 promising local almond genotypes in four Moroccan localities under two different agro-ecological systems, mountains and oasis ecosystem, was assessed by physiological means including pollen tube growth and fruit set after self- and cross-pollination and open-pollination. All studied genotypes are self-incompatible. The analysis of variance showed significant effects of the genotype, the year and the origin on fruit set in open-pollinated branches. Over two years, fruit set ranged from 5.3 % to 25.7 %, which is lower than the 30% threshold for a commercial crop in almond. The reciprocal cross-pollination test carried out in 6 genotypes from Agdez oasis locality showed that the genotypes Km-3 and Km-4 are cross-incompatible. Fruit set after cross-pollination among genotypes was low, with low to medium number of pollen tube at the style base after the reciprocal crosses, probably due to the inbreeding effect. In some crosses, the number of pollen tubes at the style base was high, whereas the fruit set was low. These results indicate that in traditional almond orchards, based on local cultivars propagated locally by seed, potential yield of the tree is limited by the effect of inbreeding depression.

Keywords: Almond, Cross pollination, Fruit set, Self-incompatibility, Inbreeding depression

INTRODUCTION

Almond (*Prunus amygdalus* Batsch syn *Prunus dulcis* (Mill.) D.A. Webb) is a deciduous fruit tree showing well adaptation to different climatic conditions ranging from moderate-winter climates where almonds have a low chilling requirement, to cold winter climates where only cultivars with high chilling requirement can be grown and produce (Socias i Company *et al.*, 2008). The introduction of the almond to North Africa came from about 500 and 600 with the arrival of the Arabs to the North Africa who brought almonds into Morocco (Lansari *et al.*, 1994). In Morocco, two categories of almond cultivation are distinguished: a traditional system dominated by local genotypes disseminated by seed and a modern system dominated by foreign cultivars, mainly 'Marcona' and 'Ferragnès', propagated by grafting. The modern system is localised mainly in Central (Saïs valley) and Central-South of Morocco (El Houz Valley) under a semi-intensive system (Mahhou and Denis, 1992). The mean yield obtained in this category is of around 0.63 Tonnes/Ha (Kodad *et al.*, 2010). The traditional system of production is located in mountain areas and in oasis and valleys in the South and Central-East of Morocco (Mahhou and Denis,1992; Lansari *et al.*, 1994). In these regions, the yield is very low (< 100 kg/Ha) and is attributed to lack or absence of pollination, irrigation, pruning or training and control of pests and diseases (Kodad and Socias i Company, 2010).

In Turkey, where most traditional almond orchards are from seeds (Çağlar *et al.*, 2005), are characterized by low and erratic yield (Oğuz *et al.*, 2011). In Iran, more than 74.000 hectares of rainfed almond orchards have been raised from seed, forming 50% of almond producing orchards in Iran, are also characterized by low yield (Rahimi and Yadollahi, 2006; Rahimi, 2002). In Tunisia, Gouta *et al.* (2019) reported that almond occupies an acreage of 250.000 ha, with low yield not exceeding 350 kg ha^{-1} with most of the producing almond orchards were traditionally established. In Italy, several authors reported that most of the trees in traditional almond orchards in Sardinia region were established traditionally by seeding and offering low yield compared to other regions using grafted and selected cultivars (Agabbio *et al.,* 1984). In all these Mediterranean's producing countries, the low yield of traditional almond orchards was attributed to the use of obsolete system practices for orchards management, extremes climatic incidences (frost damages and drought) and use of unproductive plants.

With few exceptions, most traditional almond cultivars are self-incompatible (Socias i Company, 1990; Dicenta *et al.*, 1993). Self-incompatibility (SI) in higher flowering plant is defined as the ability of the flower to prevent self-fertilization by self-pollen. In almond, as well as in other *Prunus* species, the SI system is of the gametophytic type (Socias i Company *et al.*, 1976; Dicenta and Garcia, 1993) and is controlled by a single S locus with multiple alleles (Crane and Lawrence, 1929). Self-(in)compatibility and inter-compatibility can be determined by comparing the percentage of fruit set in the field after cross-pollination and self-pollination (Grasselly and Olivier, 1981; Rovira *et al.*, 1997). Also, the observations of pollen tube growth in the style have also been used to assess self and cross-(in)compatibility in almond (Dicenta and Garcia,1993).

In almond, the successful self- and cross-fertilisation of high numbers of flowers is essential for fruit production (Kodad and Socias i Company, 2006a; 2008), since the edible part of the fruit is the seed and parthenogenesis does not ensure seed formation. Mean relative fruit set in almond has been reported to be about 30% but there is large year-to year variability, ranging from 5% (Socias i Company, 1994) to 40% (Kester and Griggs, 1959). Actually, it is difficult to establish a good fruit set for a commercial almond yield because of the large variation in bloom density among genotypes and cultivars (Socias i Company, 1988; Kodad and Socias i Company, 2008). Thus, a low fruit set in a genotype with a high bud density may give a higher yield than a high fruit set in a genotype with a low bud density (Socias i Company *et al.*, 2004). Williams (1970) reported that fruit set is the main factor determining the final fruit production. However, Dicenta *et al.*, (2006) suggested that fruit set is not a good indicator of a cultivar production, because several other factors could affect its expression. The ability of the flowers to be pollinated and fertilized depends both on the internal and external conditions. The internal factors are the genetic control of incompatibility, efficiency of pollination and the amount of flower sterility (Socias i Company, 1983; Kodad and Socias i Company, 2008) and inbreeding effect (Kodad and Socias i Company, 2008; Ortega *et al.*, 2010). The external factors are the climatic and growing conditions controlling an efficient pollination (Kester and Griggs, 1959).

The present work aims to evaluate the fruitfulness in a set of local almond genotypes by physiological mean including pollen tube growth and fruit set after self- and cross-pollination and open-pollination and to identify the causes of consistent low yields characterizing traditional almond orchards in Morocco.

MATERIALS AND METHODS

Plant materials

Twenty-six local almonds orchards were selected in four different regions having a wide range of genetic resources: Al Hoceima in the Rif Mountains (North of Morocco), Azilal in the high Atlas Mountains (South Central Morocco) and two locations of southern Morocco: Agdz oasis in the Draa valley, the Skoura oasis in the Dades valley (Figure 1). The bioclimatic category of Agdez and
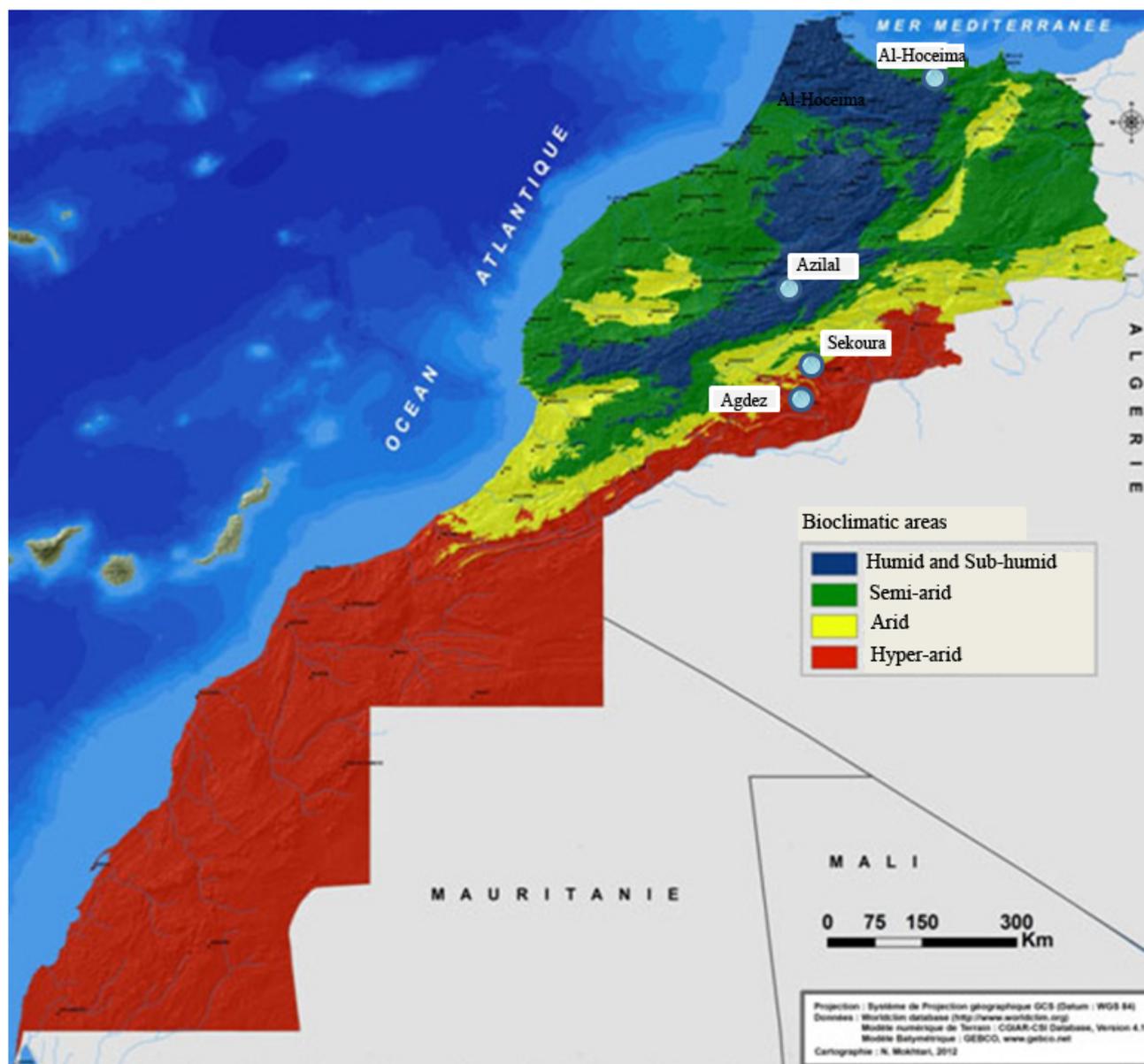

Figure 1: Bioclimatic map of Morocco with the location of the almond germplasm evaluated in this study (Adapted from Mokhtari et al., 2014)

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Skoura are classified as hyper-arid, that of Al-Hoceima as Semi-arid and that of Azilal as Humid (Mokhtari *et al.*, 2014).These genotypes were selected because of their high agronomical performance and fruit Quality (Kodad et al., 2015) (Table 1). The origin of these cultivars is open pollinated orchards. These genotypes were marked and the pollination trial was carried out during two consecutive years (2018 and 2019).

Experimental design

The self-compatibility and inter-compatibility of the trees were determined just when the first flowers were open by the observation of fruit set after pollination in the field and pollen tube growth in the laboratory. These methods had been shown to be efficient for the pollen flower compatibility evaluation (Ortega *et al.*, 2004; Socias i Company *et al.*, 2014). Self-compatibility assessment was done for all genotypes; however, the evaluation of pollen inter-compatibility was carried out only in genotypes from oasis regions. For pollen tube growth, various branches around canopy were selected and assigned arbitrarily to self and cross-pollination treatments in the field. At the first day, the flowers at stage D (Felipe, 1977) were emasculated and 2 days after were hand-pollinated on a branch of the trees studied.

The pollen was extracted from flower buds and left to dry at air temperature during 24 hours. After 24 h of pollination, the marked branches were cut from the trees and placed in plastic bags to take to the laboratory. Wet cotton was placed over the cut wound to keep the shoots humid. Once in the laboratory, the pistils were removed from flowers, placed in trays with tap water, and finally left at controlled temperature of 22°C. After 96 h after pollination, a period considered sufficient to the pollen tubes to reach the style base, the pistils were collected from the trays and were autoclaved in a 5% solution of sulphite sodium at 1.2 kg cm^{-2} during 12 min (Socias i Company *et al.*, 1976). The samples were maintained at 2–4 °C until measurements were made.

Fruit set assessment

Various twigs around canopy were selected and assigned at random to open-pollination and artificial pollination treatments to determine fruit set percentage. Three treatments were applied: open pollination as control treatment and self-pollination and reciprocal cross-pollination among genotypes. The bud flowers at stage D were emasculated and pollinated with pollen obtained from flowers buds by removing the anthers and drying them at ambient temperatures. The experimental unit

Table 1: Main trait of trees and kernel of the studied Moroccan almond cultivars

Geno-type	Blooming time	Flower bud[†] density (bud/cm)	Self compat-ibility	Harvest time	Kernel Weight (g)	Shelling percent-age	Shell hardness	Kernel taste
AT-1	Intermediate (Second decade of February)	0.54	SI	Early-July	0.97	22.2	Hard	Sweet
AT-2	Intermediate (Second decade of February)	0.76	SI	Early-July	0.95	21.7	Hard	Intermediate
AT-3	Intermediate (Second decade of February)	0.51	SI	Early-July	1.07	19.1	Very Hard	Sweet
AT-4	Intermediate (Second decade of February)	0.63	SI	Early-July	1.12	23.3	Hard	Sweet
AT-5	Intermediate (Second decade of February)	0.71	SI	Early-July	1.18	21.2	Very Hard	Sweet
AT-6	Intermediate (Second decade of February)	0.49	SI	Early-July	1.08	20.8	Very Hard	Sweet
AT-7	Late (Last decade of February)	0.63	SI	Early-July	1.18	18.9	Very Hard	Intermediate
AZ-1	Intermediate (Second decade of February)	0.67	SI	August	0.96	19.9	Very Hard	Intermediate
AZ-2	Intermediate (Second decade of February)	0.73	SI	August	0.83	22.6	Hard	Sweet
AZ-3	Late (Beginning of March)	0.72	SI	August	0.92	21.5	Hard	Sweet
AZ-4	Late (End of February)	0.63	SI	August	0.91	23.9	Hard	Sweet
AZ-5	Late (End of February)	0.56	SI	August	0.94	21.1	Hard	Sweet
AZ-6	Late (Beginning March)	0.54	SI	August	1.02	22.7	Hard	Sweet
H-1	Very Early (last January)	0.48	SI	Early-July	0.92	18.8	Very Hard	Sweet
H-2	Very Early (last January)	0.62	SI	Early-July	1.07	23.6	Hard	Sweet
H-3	Early (Beginning of February)	0.58	SI	Early-July	1.03	22.6	Hard	Sweet
H-4	Very Early (last January)	0.55	SI	Early-July	0.92	25.6	Hard	Sweet
H-5	Very Early (Last January)	0.68	SI	Early-July	0.88	18.5	Very Hard	Sweet
H-6	Early (Second decade of January)	0.61	SI	Early-July	0.96	22.6	Hard	Sweet
KM-1	Intermediate (Second decade of February)	0.67	SI	End-July	1.01	21.3	Hard	Sweet
KM-2	Late (Last decade of February)	0.73	SI	End-July	1.07	21.5	Hard	Sweet
KM-3	Late (Early March)	0.57	SI	End-July	1.05	22.1	Hard	Sweet
KM-4	Intermediate (Second decade of February)	0.54	SI	End-July	1.03	19.3	Very Hard	Sweet
KM-5	Intermediate (Second decade of February)	0.56	SI	End-July	0.97	19.2	Very Hard	Intermediate
KM-6	Intermediate (Last decade of February)	0.64	SI	End-July	0.94	22.8	Hard	Intermediate
KM-7	Late (Early March)	0.62	SI	End-July	1.12	30.0	Semi Hard	Sweet

Flower bud density was determined according to Kodad and Socias i Company (2006b).

was a branch and four replications per treatment were adopted. The fruit set percentages were determined on open-pollination branches as the number of fruit set/initial bud number. For the artificial pollination treatment, fruit set percentages were determined as the number of fruit set in relation to the number of pollinated pistils. The fruit sets evaluation on open-pollinated twigs was assessed during two consecutive years (2018 and 2019).

Microscopic observation

The pistils were prepared according to Socias i Company et al. (1976), by dissecting the outer part of each pistil and leaving only the tissue through which the pollen tubes grow. Tube pollen growth was assessed by observation in an UV illumination microscope (Ortholux II, Ernst Leitz GmbH, Wetzlar, Germany) provided by mercury lamp (Osram HBO 200 W/4). Fluorescence of the callose deposits in the pollen tubes following aniline blue-staining after squashing the pistils (Linskens and Esser, 1957) was used for visualization. In the present study, the number of pollen tubes at the base of each style or in the ovary was assessed in order to evaluate the self-compatibility of each genotype and inter-compatibility among genotypes.

Statistical analysis

Data were analysed using the General Linear Model procedure of the SAS 2000 programme (SAS Institute, Cary, NC, USA). The procedure PROC GLM was used for the analysis of variance. Mean separations were analysed by Duncan's multiple range test at $P < 0.05$.

RESULTS AND DISCUSSION

Fruit set after open pollination

The studied genotypes are characterized by flowering date ranging from very early to late and harvesting time from Early July to August (Table 1). The nut of these genotypes is hard to very hard and the kernel weight varied between 0.83 g to 1.18 g, with good and sweet taste (Table 1). Fruit set in open-pollinated branches was assessed over two consecutive years to evaluate the potential productivity of all genotypes. The statistical analysis showed significant effects of the genotype, the year and the geographical origin on fruit set (Table 2). Fruit set ranged between 5.28 % for Km-4 and 24.4 % for Az-5 in 2018, and 9.65 % for Km-4 and 25.7 % for Az-6 in 2011 (Table 3). Fruit set did not reach 30% in any selection during the

2 years (Table 3), the threshold considered for a commercial crop (Kester and Griggs, 1959). Similar results were reported in other almond genotypes (Kodad and Socias i Company, 2006a; 2008). Fruit set in almond is strongly influenced by the presence of pollinizer cultivars, insect pollinators and by climatic conditions affecting pollen viability, germination and pollinator activity (Gradziel and Weinbaum, 1999; Kozlowski and Pallardy 2002; Ortega *et al.*, 2007; Tombesi *et al.*, 2017). In the present study, the genotypes were self-incompatible and grown under marginal conditions without the presence of a pollinator and lack of adequate crop management (lack of adequate irrigation, fertilization and chemical treatments to control pests and diseases). These growing conditions could affect the fruit set in these local almond genotypes. Dicenta *et al.*, (2006) reported that fruit set does not seem to be a good indicator of production capacity of the tree,

Table 3: Fruit set percentage in 26 almond genotypes over 2 years of study

Geno-type	Geographi-cal origin	Years		Mean
		2018	**2019**	
AT-1	Agdez	10.1 jkl[z]	18.3 bcdefg	14.2 fghij
AT-2		11.1 hijk	14.3 ghkij	12.7 hijk
AT-3		9.97 ikl	10.0 k	10.0 jkl
AT-4		12.8 ghijk	18.4 cdefgh	15.6 efgh
AT-5		9.2 kl	11.4 ijk	10.3 ijkl
AT-6		8.42 kl	9.69 k	9.05 kl
AT-7		18.1 bcdefg	17.1 defghi	17.6 bcdef
AZ-1	Azilal	18.1 bcdef	22.5 abc	20.3 abcdef
AZ-2		18.7 bcdef	22.2 abcd	20.4 abcd
AZ-3		10.8 ijk	17.1 cdefghi	13.9 fghij
AZ-4		18.9 bcdef	15.6 fghij	17.2 cdef
AZ-5		24.4 a	20.2 abcdefg	22.3 abc
AZ-6		22.0 abc	25.7 a	23.9 a
H-1	Al-Hoceima	16.6 defg	18.8 hijk	17.7 dfghi
H-2		14.6 fhgij	21.6 abcde	18.1 bcdef
H-3		18.6 bcdef	22.1 abcd	20.3 abcde
H-4		17.9 bcdefg	22.5 abc	20.2 abcde
H-5		15.6 efghi	20.4 abcdef	18.0 bcdef
H-6		20.9 abcde	14.6 eghijk	17.8 bcdef
KM-1	Sekoura	13.1 ghijk	10.1 jk	11.6 hijkl
KM-2		23.0 ab	16.4 defghi	19.7 abcde
KM-3		8.43 kl	10.1 k	9.27 kjl
KM-4		5.28 l	9.6 k	7.46 l
KM-5		17.6 cdefg	15.9 defghi	16.7 def
KM-6		16.0 defgh	14.3 ghijk	15.2 efghi
KM-7		21.3 abcd	23.7 ab	22.5 ab

[z] *Different lower case letters in the same column indicate significantly different values per p <0.05.*

Table 2: Analysis of variance for fruit set percentage after open pollination in all studied almond genotype over two seasons

Source of Variation	df	Means squares [†]	F-value	p
Year	1	47.2 **	8.48	0.0053
Geographical origin	3	256.7 ***	46.1	<.0001
Year × Geographical origin	3	14.7 *	2.64	0.0492
Genotype (Geographical origin)	22	58.0 ***	10.4	<.0001
Year × Genotype (Geographical origin)	22	16.7 ***	3.03	0.0005
Error	52	5.57		

[†]*, **, ***: Significance of the mean squares at P < 0.05 P< 0.01 or P< 0.001 by the t-test, respectively.

but a consequence of several factors such as flower density and quality (Socias i Company *et al.*, 2004; Kodad and Socias i Company, 2006a). The evaluation of flower bud density of the present study showed that the values varied between 0.48 bud/cm and 0.76 bud/cm (Table 1), similar to those of commercial almond cultivars (Kodad and Socias i Company, 2006a; 2008). Several studies reported that when the number of flower buds was reduced, the fruit set was increased (Dicenta *et al.*, 2006; Egea *et al.*, 1986). Thus, the values of the flower buds density of the studied genotypes might not explain the consistent low fruit set obtained in some genotypes (KM-3, KM-4, AT-3, AT-5 and AT-6).

In the present study, fruit set was significantly higher in 2019 (17.0 %) than in 2018 (15.6 %) (Table 4). The year to year variation of fruit set has often been described for almond, independently of self-(in)compatibility of the genotype (Socias i Company et al.,2005; Kodad and Socias i Company, 2014; Dicenta *et al.*, 2006), confirming that almond is particularly prone to erratic fruit production. In our study, the effect of the location was revealed to be significant for fruit set (Table 2). The mean value of fruit set of genotypes from Skoura and Agdez (oasis pool) showed the lowest fruit set in both years of study, whereas those from Azilal showed the highest mean values (Table 4). In all these locations, no spring frosts took place during the blooming period in both years of study, with a mild winter, that could damages flowers and reduce the fruit set, taking into account that fruit set obtained in the four regions were lower than 30%, the threshold considered for a commercial crop (Kester and Griggs, 1959). Thus, the consistent low fruit sets obtained in oasis pool could be due to other unidentified environmental and genetic factors. Similar results were reported for apricot genotypes grown under similar growing conditions (Kodad *et al.*, 2013).

Table 4: Average fruit set percentage after open pollination treatment according to geographical origin and year

Geographical origin	Years		
	2018	**2019**	**Mean**
Agdez	11.4 d [z]	14.2 c	12.8 d
Sekoura	14.9 c	14.3 c	14.6 c
Al-Hoceima	17.4 b	19.2 b	18.3 b
Azilal	18.8 a	20.5 a	19.7 a
Mean	15.6	17.0 [†]	

[z] *Different lower case letters in the same column indicate significantly different values at p <0.05.*
[†] *Mean differences between year significant at p<0.5*

Self-compatibility evaluation

The microscopic observation of the pollen tube growth in the style showed that pollen tubes were stopped in the upper part of the style and no pollen tubes were found entering the ovary and pollen tube growth stopped near the style base (Table 5). These results agree with those observed in several self-incompatible almond cultivars (Dicenta *et al.*, 1993; Kodad and Socias i Company, 2008; Ortega *et al.*, 2005). These results also coincide with the null fruit set obtained after self-pollination in

the field (Table 5), and clearly indicate that these genotypes were self-incompatible. During the two years of study, no adverse climatic conditions (frost) that could damages flowers and fruit set were observed. Thus, all these local genotypes selected from different regions are self-incompatible. Recently, El Hamzaoui *et al.*, (2015) determined the S-alleles of 70 local almond genotypes from different region of Morocco grown in the collection of Meknes and reported that almost of these genotypes have the S-allele responsible of self-incompatibility. However, three of them share the S_f-allele responsible of self-compatibility expression in almond (Bošković *et al.,* 1999). However, no studies were carried out to determine the self-compatibility phenotype of these genotypes by microscopic observation of pollen tube growth and fruit set after self-pollination.

Table 5: Fruit set, percentage of pistils with pollen tube and number of pollen tube at their base (%) after self- and cross-pollination treatment in 6 genotypes from Agdez oasis

Cross	Fruit set (%)	Percentage of pistils with pollen tube at style Base (%)	Number of pollen tube at the style Base
KM1 × KM1[z]	0	0	0
KM1 × KM2	3.76 h[z]	27.3 q	1 k
KM1 × KM3	15.1 bcdef	80.0 i	3.13 cdef
KM1 × KM4	8.48 gh	63.6 l c	1.40 ijk
KM1 × KM5	24.6 abcd	91.7 p	3.82 bc
KM1 × KM6	7.35 gh	36.4 p	1.20 jk
KM2 × KM2 [z]	0	0	0
KM2 × KM3	19.6 abcdef	83.3 g	2.01 jk
KM2 × KM4	9.11 fgh	41.7 o	1.40 hij
KM2 × KM5	26.4 a	84.6 f	3.18 ijk
KM2 × KM6	3.74 h	58.3 m	1.57 cde
KM3 × KM1	13.6 defgh	63.6 l	1.89 ijk
KM3 × KM2	13.8 defgh	72.7 j	2.25 hijk
KM3 × KM3[z]	0	0	0
KM3 × KM4[z]	0	0	0
KM3 × KM5	25.6 ab	81.8 h	3.13 cdef
KM3 × KM6	14.3 cdefg	66.7 k	2.13 ghij
KM4 × KM1	12.3 fgh	83.3 g	1.56 ijk
KM4 × KM2	6.78 gh	54.5 n	1.83 hijk
KM4 × KM3[z]	0	0	0
KM4 × KM4[z]	0	0	0
KM4 × KM5	20.1 abcdef	83.3 g	3.20 cd
KM4 × KM6	12.5 efgh	90.9 d	1.50 ijk
KM5 × KM1	19.9 abcdef	90.9 d	3 cdefg
KM5 × KM2	21.7 abcde	91.7 c	2.91 efgd
KM5 × KM3	21.8 abcde	83.3 g	2.50 defgh
KM5 × KM4	23.1 abcde	100 a	2.90 defg
KM5 × KM5[z]	0	0	0
KM5 × KM6	21.0 abcde	92.3 b	4.64 a
KM6 × KM1	5.19 gh	41.7 o	1.20 jk
KM6 × KM2	9.11 fgh	63.6 l	1.57 ijk
KM6 × KM3	8.47 gh	83.3 g	2.20 h
KM6 × KM4	7.11 gh	80.0 i	2.2 ghi
KM6 × KM5	25.1 abc	90.0 e	4.11 ab
KM6 × KM6[z]	0	0	0

[z] *These crosses are not included in the statistical analysis. Different lower case letters in the same column indicate significantly different values at p <0.05.*

Cross-pollination test

The cross-pollination assay was conducted in 6 genotypes from the Agdez oasis because of their consistent low fruit set over two years of study in order to verify if it is due to the inter-incompatibility among genotypes or to the inbreeding effect, which was reported to affect negatively the pollen tube growth in pistil (Alonso and Socias i Company, 2005), the embryo sac development and fruit abortion (Ortega *et al.*, 2010; Martinez Garcia *et al.*, 2011; 2012). The effects of the pollen source (male) and pistils (female), independently of the reciprocal crosses, were statistically significant on the fruit set, percentage of the styles and number of pollen tube at the style base (Table 6). Firstly, the microscopically observation of the stigmata revealed that in all crosses the values of germinated pollen grains on the stigmata was over 50, considered the minimum to avoid any mass effect of pollen load, thus attributing the differences to the (in)compatibility degree (Kodad and Socias i Company, 2006b). Moreover, no significant correlation was found between the number of pollen tubes at the style base and fruits set, showing the independence of both variables (Kodad and Socias i Company, 2008; Ortega *et al.*, 2010). The results showed that no fruit set was obtained after reciprocal cross of the genotypes Km-3 and Km-4 (Table 5). The microscopic observation showed that the pollen tubes were arrested in the upper part of the style and no pollen tubes were observed at the style base after cross pollination of these genotypes (Table 5). Thus, these genotypes are inter-incompatible and probably share the same S-alleles. However, for the others genotypes, the fruit set, unlike the reciprocal cross, varied between 2.73 % and 26.4 (Table 6), indicating that these genotypes are inter-compatible.

Table 6: Percentage of pistils with pollen tube and Number of pollen tube at the style base (%) for different cross-pollination treatments

Geno-type	Fruit set (%)	Percentage of pistils with pollen tube at style Base (%)	Number of pollen tube at the style Base
Pollen behaviour			
KM-1	10.1 c	63.2 d	1.95 c
KM-2	11.0 c	62.0 d	2.15 bc
KM-3	16.3 b	82.5 b	2.42 b
KM-4	12.0 bc	71.3 c	2.18 bc
KM-5	24.4 a	86.3 a	3.49 a
KM-6	11.8 bc	68.9 c	2.43 b
Pistils behaviour			
KM-1	11.9 c	59.8 d	2.6 b
KM-2	12.5 bc	60.9 d	2.11 c
KM-3	16.8 b	71.2 c	2.33 bc
KM-4	12.9 bc	78.0 b	2.05 c
KM-5	21.5 a	91.6 a	3.21 a
KM-6	11.0 c	71.7 c	2.42 b

[z] Different lower case letters in the same column indicate significantly different values at p <0.05.

The pistil effect was significant on the studied variable (Table 5). The highest mean value of fruit set was obtained for genotype Km-5 (21.5 %) pollinated by the others pollen, followed in decreasing degree by that of Km-3 (16.8 %), Km-4 and Km-2 (12.5 %) and finally by Km-6 (11.0 %) (Table 6). The values ranged between 19.9 % and 23.1 % for the crosses Km-5 × Km-1 and Km-5 × Km-4, respectively (Table 5). On the other hand, the percentage of style with pollen tube at their base after cross pollination showed that the highest values were observed in the Km-5 pistils (91.6 %), with the highest mean number of pollen tube at style base (3.21) (Table 6). The pollen effect was significant on the studied variable (Table 6). The highest values of fruit set, the percentage of the pistils and number of pollen tubes at their style base were obtained with the pollen of the genotype Km-5, followed by that of Km-3 (Table 6). However, the others genotypes showed variable and approximately similar values (Table 6). The pollination by the pollen of Km-5 resulted in high fruit set for all studied genotypes (Table 6), with value ranged between 20.0 % for the cross Km-6 × Km-5 and 26.4 % for the cross Km-3 × Km-5 (Table 5). For the genotype Km-3, when was used as female, the highest fruit set was obtained after pollination by the pollen of Km-5 (25.6%), followed in decreasing degree by km-6 (14.2 %), Km-2 (13.7 %) and finally Km-1 (13.1 %) (Table 5). When this genotype was considered male, the highest value of fruit set was obtained after pollination of Km-5, and the lowest value was found with Km-1 (Table 5). The percentage of the pistils with pollen tube at the style base of Km-3 was higher when it was pollinated by the pollen of Km-5 (81.8%), followed in decreasing degree by that of Km-2 (72.7%), Km-6 (66.7%) and Km-1 (63.6 %) (Table 6). Similarly, the highest number of pollen tubes at the style base of Km-3 pistils was observed when pollinated by Km-5 (3.12), whereas the lowest value was obtained by the pollen of Km-1 (1.88) (Table 5). Thus, the pistils of km-3 could be considered able to sustain the pollen tube growth because of the high percentage of the style with pollen tube at their base (when pollinated by Km-5 and Km-2), with high number of pollen tube (≥1.9), indicating that low fruit set obtained on open pollination branches over two years of study (10.0 %) and after cross pollination with Km-1, Km-2 and Km-6 might not be due to a deficient reserve accumulation in the pistil transmitting tissue (de Graaf *et al.*, 2001), required to sustain the pollen tube growth to reach the base of style and to penetrate into the ovary.

Concerning the reciprocal crosses among the genotypes Km-1, Km-2, Km-4 and Km-6, the fruits set was low (<13%). Similarly, the percentage of pistils with pollen tube at the style base were low after reciprocal crosses, with the exception of km-4 × km-1, Km-4 × km-6 and Km-4 × km-6 crosses (Table 5). In the same way, the number of pollen tube at the style base were low (<1.6), unlike the reciprocal cross, excepting in km-4 × km-2 and km-6 × km-4 crosses (Table 5). Also, it has been reported that the pollination failures after self-pollination was attributed to the pistil–pollen interaction in inbred pistils, where a negative effect for related pollen could be

activated (Alonso and Socias i Company, 2005b). This negative reaction was reported to be expressed against self-pollen and also against related pollen with the same parental origin (Alonso and Sociasi Company, 2005a). In almond, it has been reported that the ovule fertilization may take place with a single pollen tube entering the ovary (Pimienta and Polito, 1983), even if a high number of pollen tubes reaching the style is suitable to increase a successful fertilization (Alonso and Socias i Company, 2005b).

All these results indicate that low fruit set obtained on branches after open pollination and reciprocal cross of genotypes Km-1, Km-2, Km-4 and Km-6 could be due to the failure of fecundation as result of inbreeding effect that probably characterize most local almond population in Morocco because in these regions the almond trees are propagated by seed collected from a small number of genotype showing high production. Consequently, the genetic pool in these area has been gradually reduced, mainly in isolated and diminutive sites where almond is cultivated such in oasis ecosystem, leading to the emergence of inbreeding phenomenon. Indeed, the crosses between genetically related genotypes could generate inbreeding effects in the progeny (Socias i Company and Felipe, 1988; Lansari *et al.*, 1994; Ortega and Dicenta, 2003). Inbreeding is a genetic particularity giving arise to the expression of lethal and deleterious genes, which could affect negatively different morphological, physiological biochemistry process of the tree (Socias i Company, 2002; Ortega and Dicenta, 2003; Alonso and Socias i Company, 2005a). In self-compatible inbred genotypes, the fruit set was reported to be lower after self-pollination than after cross-pollination with unrelated pollen (Socias i company and Alonso, 2004). However, several studies point out that the low fruit set obtained after self-pollination of inbreed genotypes seems to be a direct consequence of endosperm degeneration during fruit development, caused by inbreeding depression (Ortega *et al.*, 2010; Martinez Garcia *et al.*, 2011; 2012).

CONCLUSION

The present study showed that the fruit set after open pollination of most selected Moroccan local almond cultivars grown in their original areas were consistently low (<25%) over two years of study, with values lower than the threshold level considered for commercial crop. These results could explain in part the low yield observed in traditional almond production sector characterized by high level of co-ancestry among the cultivars and genotypes cultivated in this area that they are propagated by seeds collected from narrow set of genotypes with high yield and physical fruit quality. The evaluation of the pollen tube growth in the style and fruit set after self-pollination showed that all tested genotypes are physiologically self-incompatible. Some genotypes are physiologically cross-incompatibles after reciprocal cross-pollination. The study of pollen tube growth in the style and fruit sets after reciprocal cross-pollination test, among genotypes from Agdez oasis ecosystem, re-

vealed that the fruit sets were lower than excepted and the number of pollen tube at the style base was low to medium. Moreover, in some crosses, the number of pollen tube at the style base was high, whereas the fruit set was low. These results point out to the possible effect of inbreeding on the fruit set observed in these genotypes through the reduction of the number of pollen tubes at the style and probably by endosperm degeneration during fruit development. The present results indicate that in traditional almond orchards, based on the local cultivars propagated by seeds, the potential yield of the tree is limited by the effect of inbreeding depression. Thus, in addition to the growing conditions, technical handling and self-incompatibility trait, other factors must be taken into account when evaluating yield in traditional producing regions where plants are propagated by seeds, such as the inbreeding effect and cross-incompatibility among the cultivars and genotypes.

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