

Selection of potential pollinizers for 'Hass' avocado based on flowering time and male–female overlapping

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ABSTRACT

Avocado production is dependent on the singular synchronous protogynous dichogamy of the species that promotes outcrossing. With the objective of selecting potential pollinizer avocado genotypes for 'Hass', the most important avocado cultivar worldwide, we have monitored during two consecutive years the flowering phenology of 27 avocado genotypes in South-eastern Spain. The average length of the flowering season was 45 days ranging from 18 days for 'Harvest' to 50 days for 'Fuerte'. The earliest genotypes to flower were 'Fuerte' and 'Shepard' that started blooming during the third week of March. The latest genotypes to flower were 'Colin V-33', 'Adi', 'OA184' and 'Harvest', which started blooming in the second week of April. 'Hass' blooming lasted 30 days, from the first week of April until the second week of May. Since a good pollinizer must present not only an overlapping in the flowering season but also an overlapping in sexual stages with the pollinated cultivar, a group of 12 genotypes ('Hass', 'Fuerte' and 10 genotypes producing 'Hass-like' fruit with good overlapping in the flowering season with 'Hass') was studied with more detail determining daily the stages of male and female overlapping every 2 h. Results herein indicate that 'Marvel' and 'Nobel' showed a high sexual overlapping with 'Hass'. Taking into account the flowering phenology, the overlapping in sexual stages and the fruit set obtained with hand-pollinated flowers in the field, those two genotypes could be an interesting alternative to the current use of 'Fuerte' as pollinizer for 'Hass' in South-eastern Spain.

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1. Introduction

Avocado (*Persea americana* Mill.) is an evergreen subtropical fruit tree native to Central America and Mexico where it was domesticated and cultivated from ancient times (Galindo-Tovar et al., 2008; Chen et al., 2009). It is a member of the Lauraceae, a mostly subtropical or tropical family included in the basal angiosperm clade Magnoliid within the order Laurales. Several well-defined geographical ecotypes or botanical varieties of avocado adapted to different environmental conditions have been described of which three are considered of horticultural interest: Mexican [*P. americana* var. *drymifolia* (Schlecht. & Cham.) Blake], Guatemalan (*P. americana* var. *guatemalensis* L. Wms.) and West Indian (*P. americana* var. *americana* Mill.). These botanical varieties are distinguishable on the basis of morphological, physiological and horticultural traits (Bergh, 1995; Bergh and Lahav, 1996; Chanderbali et al., 2008). Thus, while Mexican and Guatemalan races are adapted to cooler climates, the West Indian race requires a warmer climate for optimum development. Most commercial avocado cultivars are interracial hybrids developed from chance

seedlings. The most widely grown cultivar worldwide is 'Hass', a black-skinned Guatemalan × Mexican hybrid.

Total world avocado production has reached more than 3.3 million tons in 2007, with a few countries (Mexico, Indonesia, USA, Colombia, Brazil, Chile, Dominican Republic and Peru) accounting for more than 70% of that production with Mexico being the main avocado producing country with more than 1 million tons and 30% of total world production (FAOSTAT, 2008). Spain is a singular case in avocado cultivation since it is the only European country with a significant commercial production (about 85,000 tons in 2007, FAOSTAT, 2008) limited to the Southern Mediterranean coast of Andalucía and the Canary Islands.

Avocado has a synchronous protogynous dichogamous breeding system that promotes outcrossing. Each perfect flower opens twice, the first functionally as a female flower with a white receptive stigma; then the flower closes and the following day the flower reopens functionally as a male flower with the stigmas no longer receptive and dehisced anthers (Davenport, 1986). The different avocado cultivars are classified in two groups (A or B) based upon their flowering behaviour (Nirody, 1922). In the type A cultivars, the flowers open in the morning in the female stage, close at mid-day and reopen the afternoon of the following day at the male stage. In the type B cultivars, the flowers open in the afternoon at the female stage, close in the evening and reopen the

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following morning at the male stage (Stout, 1923). The combination of protogynous dichogamy and the flowering behaviour of the avocado prevents self-pollination promoting outcrossing. However, self-pollination occurs in orchards composed of a single genotype probably due to some overlapping between the male and female stages in the same flower, among flowers of the same tree or among flowers of different trees of the same genotype (Davenport, 1986).

A general problem of commercial avocado fruit production is the excessive flower and fruit abscission; thus, final fruit set can range from 0.001% to 0.23% (Sedgley, 1980) and a yield of 300 fruits per tree can be considered as a good production (Lahav and Zamet, 1999). Under optimum conditions, the floral behaviour is predictable. However, the flower opening cycle is sensitive to environmental conditions, mainly temperature (Lesley and Bringham, 1951; Sedgley, 1977; Sedgley and Annels, 1981; Ish-Am and Eisikowitch, 1991). The sensitivity of the flowering behaviour to environmental factors depends on the cultivar, and, in general, type B cultivars are more sensitive than type A cultivars to low temperatures (Sedgley and Grant, 1983).

In spite of the high percentage of self-pollinations reported in different environmental conditions (Davenport, 1989; Davenport et al., 1994), molecular studies of the genetic composition of the progeny obtained indicate that most of the final fruits are the result of outcrossing (Vrecenar-Gadus and Ellstrand, 1985; Degani et al., 1986, 1989, 1997, 2003; Goldring et al., 1987; Robbertse et al., 1997; Chen et al., 2007; Borrone et al., 2008). Thus, traditionally it has been considered that interplanting complementary cultivars in close proximity significantly increases yield in avocado (Bergh and Garber, 1964; Vrecenar-Gadus and Ellstrand, 1985; Degani et al., 1997) although the relative impact of outcrossing in final yield is still under debate (Garner et al., 2008).

Consequently, the existing evidence suggests that interplanting cultivars of opposite flowering types can be important to secure a good crop. However, depending on the yield and final market price of the fruit of the pollinating cultivar, profitability of the production can be reduced. The most important commercial cultivar in Spain, as in most avocado producing countries, is 'Hass' with 75% of the avocado production and 'Fuerte' is the most commonly cultivar used as pollen donor. However, 'Fuerte' is currently of less commercial interest than 'Hass', and the search for new pollinizers producing 'Hass-like' fruit can be advantageous. With the objective of selecting potential pollinizers for 'Hass', in this work the flowering phenology and daily flowering cycle of the most promising genotypes conserved in our germplasm collection is characterized under the environmental conditions of South-eastern Spain. Furthermore, those genotypes that present sufficient overlapping in the blooming period with 'Hass' and produce 'Hass-like' fruit are characterized with more detail. Finally, the effect of three potential pollinizers on fruit set is analyzed under field conditions.

2. Materials and methods

2.1. Climatic conditions

In continental Spain, avocados are grown in the Southern Andalusian coast (provinces of Malaga and Granada). Data at the E.E. la Mayora (36°45'N) for the last 45 years show an annual average of the mean temperatures of 19.4 °C with an average of 29.4 °C of the high temperatures in the hottest month (August) and an average of 9.3 °C of the low temperatures in the coolest month (January) with an annual rainfall of 435 mm.

2.2. Plant material and flowering period

This work was carried out on 27 avocado genotypes conserved in the avocado collection at the E.E. La Mayora (Málaga, Spain) comprising genotypes of both A and B types (Table 1).

During 2005 and 2006, the length of the flowering period, defined as the number of days between opening of the first flowers and closing of the last flowers, was established in at least two trees of the 27 avocado genotypes studied. Full bloom was estimated when more than 50% of the flower buds had opened.

2.3. Daily flowering cycle

The flowering cycle was studied daily in two trees of 12 genotypes: 'Hass', 'Fuerte' and 10 genotypes that produce 'Hass-like' fruit and show a high overlapping in blooming period with 'Hass' ('Gem 3-29-5', 'H670', 'Hass Motril', 'Jimenez 1', 'Jimenez 2', 'Lamb Hass', 'Lohneiss', 'Marvel', 'Nobel' and 'Tacambaro') in order to compare the overlapping between the male phase of these putative pollinizers and the female phase of 'Hass'. Every 2 h from 8:00 to 20:00 the flowering behaviour of each genotype was observed counting the flowers in male and female stage in 10 inflorescences located in different positions in the trees.

Moreover, to study the normal cycle of dichogamy under our environmental conditions in 'Hass' and three type B genotypes ('Marvel', 'Nobel' and 'Fuerte') a total of 20 flowers (10 per tree) were labelled daily during the blooming period and the day of the first opening was noted. These flowers were monitored until the second flower opening.

2.4. Pollinizer efficiency under field conditions

Hand-pollinations were carried out to compare the effect of three pollen sources on fruit set of 'Hass'. The three pollen donors selected in this study were: 'Nobel' and 'Marvel' (selected due to the results obtained during the monitoring of the flowering cycle) and 'Fuerte' (the most common cultivar used as pollen donor in Southern Spain). A total of 500 inflorescences distributed in 25 'Hass' trees were hand-pollinated. Three flowers were pollinated in each inflorescence using a different pollen donor for each one. Male flowers were collected at 12:00 and the pollen was immediately applied on the stigma of 'Hass' female flowers by direct contact of

Table 1

List of the 27 avocado varieties studied to determine the blooming phenology. Superscripts indicate the reference where the flower group was studied.

Genotype	Flower group	Genotype	Flower group
5-552	B ^d	Lamb Hass (BL122)	A ^a
Adi	A ^b	Lohneiss	A ^a
Colin v-33	B ^b	Marvel (BL516)	B ^b
Eden	A ^c	Negra de la Cruz	B ^b
Fuerte	B ^a	Nobel (BL667)	B ^b
Fundacion II	A ^a	OA184	B ^g
H670	A ^a	Pinkerton	A ^b
Gem (3-29-5)	A ^b	Regal	A ^c
Harvest	A ^d	Rincoatl	A ^b
Hass	A ^a	Rt 5176	B ^c
Hass Motril	A ^a	Shepard	B ^f
Iriet	B ^b	Sir Prize	B ^b
Jimenez 1	A ^a	Tacambaro	A ^a
Jimenez 2	A ^a		

^a This work.

^b <http://www.ucavo.ucr.edu>.

^c Lavi et al. (1997).

^d Arpaia et al. (2005).

^e Martin and Bergh (1993).

^f Newett et al. (2002).

^g Witney and Martin (1995).

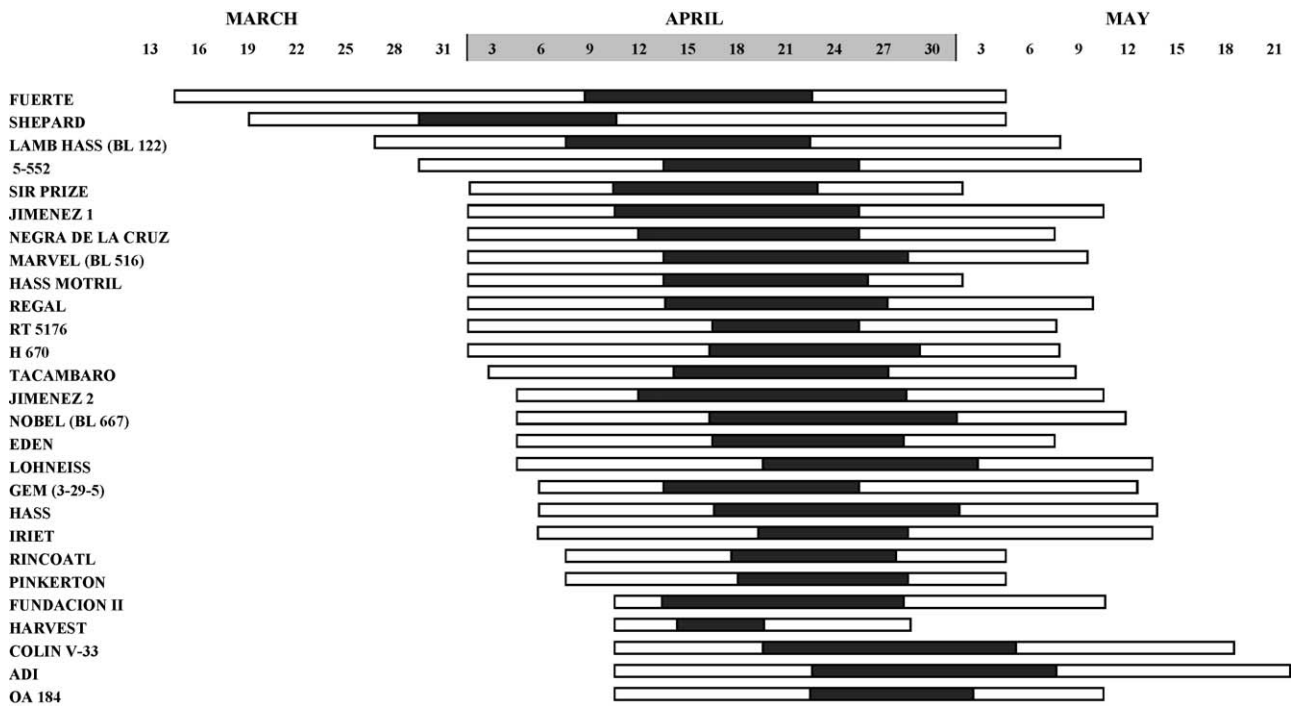


Fig. 1. Diagram of flowering of the 27 avocado genotypes studied in this work. Full bloom is showed in dark colour. Data are mean values of two years observations.

the anthers with the stigma. The rest of the non-pollinated flowers in the inflorescences were removed. The number of flowers and developing fruits retained by the trees were monitored weekly, the abscission date was noted and the final fruit set was calculated. Proportion comparisons were analyzed using Yates' chi-square goodness-of-fittest in 2 × 2 contingency tables testing for association between pollen donor genotypes and fruit set. The SPSS package v 15 (SPSS Inc., Chicago, IL) was used for all statistical analyses.

3. Results and discussion

3.1. Flowering phenology

The study of flowering phenology during two consecutive years showed that the length of the blooming period in the genotypes studied ranged from 18 days in 'Harvest' to 50 days in 'Fuerte' and full bloom varied from one to two weeks in most genotypes (Fig. 1). The length of the blooming period for each genotype was similar during the two years studied, although in 2006 the beginning of flowering occurred one week earlier than in 2005. We could distinguish several early flowering genotypes ('Fuerte', 'Shepard' and 'Lamb Hass'), whose flowers start opening in March, and late flowering genotypes ('Harvest', 'Colin V-33', 'Fundacion II', 'OA184' or 'Adi'), whose flowers start opening at mid-April.

Full bloom for most genotypes occurred during April, displaying a good overlapping with 'Hass' (Fig. 1). However, full bloom of 'Fuerte', the most commonly used pollinizer for 'Hass' in Spain, only showed a good overlapping with 'Hass' during the first half of 'Hass' full bloom; consequently it will be of interest to find additional pollinizers for the second half of the 'Hass' flowering period.

3.2. Daily flowering cycle

The daily flowering cycle has been monitored in 'Hass', 'Fuerte', and in 10 'Hass-like' genotypes in order to study possible complementation between flower stages. Although avocado has

been described as a species with a marked protogynous dichogamy, it is common to observe flowers in different sexual stages at the same time among trees of the same genotype and even within the same tree. This overlapping is more extense at the start and at the end of the flowering season and this may help to explain fruit production in single cultivar blocks (Davenport, 1989; Davenport et al., 1994). Thus, although dichogamy enhances the opportunity of cross-fertilization and, hence, is a mechanism that reduces inbreeding, the overlapping between flowers in both male and female stages can be considered as a bet-hedging strategy to ensure fertilization and seed production when the opportunities for outcrossing are limited.

During the period of full bloom, the flowers of the type B genotypes studied ('Fuerte', 'Nobel' and 'Marvel') opened in the male stage between 8:00 and 10:00 (Fig. 2). At 12:00, the anthers

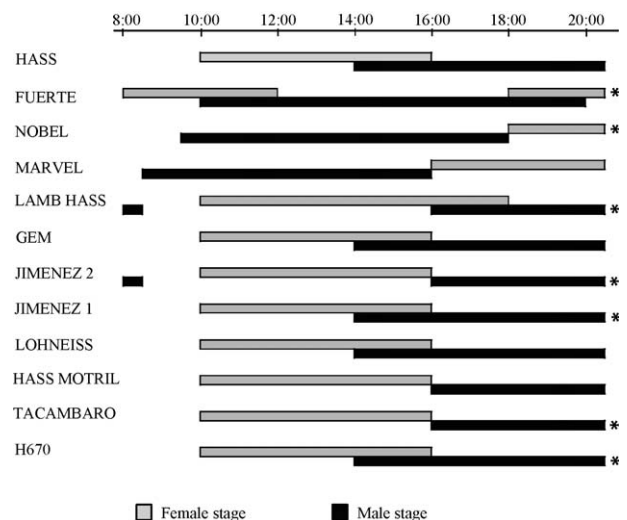


Fig. 2. Daily sexual stage of flowers of different genotypes with 'Hass-like' fruit during the full bloom period. Data are mean values of two years observations. *Flowers remain open after the last monitoring.

became dehiscent. Previous works have described that 'Fuerte' shows a high overlapping between the female and male stages within the same inflorescence (Nirody, 1922). This is also the case in this work, where 'Fuerte' showed a high overlapping between male and female flowers in the same trees from 10:00 to 12:00 and from 18:00 to 20:00, whereas no overlapping was observed in both 'Nobel' and 'Marvel'. Male flowers closed in the afternoon and female flowers closed in the evening except in the case of 'Fuerte' whose flowers opened in the female stage later and many of them continued open during the early hours of the following morning where it was common to observe overlapping between male and female flowers. During the period of full bloom, flowers of the type A genotypes studied ('Hass', 'Lamb Hass', 'Gem', 'Jimenez 1', 'Jimenez 2', 'Hass Motril', 'Lohneiss', 'Tacambaro' and 'H 670') opened as females at 10:00 and remained open until 16:00 (18:00 in the case of 'Gem'). Overlapping between closing female flowers and opening male flowers occurred in 'Hass', 'Gem', 'Jimenez 1', 'Lohneiss' and 'H670' between 14:00 and 16:00; in 'Lamb Hass' the overlapping took place between 16:00 and 18:00 whereas no overlapping was observed in 'Jimenez 2', 'Hass Motril' or 'Tacambaro', except at the end of the flowering season.

'Hass' flowering revealed a wide variation in floral behaviour at the beginning and at the end of the blooming season. Thus, at the beginning of the blooming season, flowers opened at the female stage at 10:00 and closed at 16:00 whereas they opened at the male stage at 14:00 and remained in that stage overnight beginning to close between 10:00 and 12:00. In this period, overlapping between the male and female stages could be found during 4 h: 10:00–12:00 and 14:00–16:00. When compared with 'Hass', 'Marvel' had a similar blooming season and an overlapping with 'Hass' flowers could be observed during the first morning hours, although anther dehiscence took place at 12:00. At the end of the blooming period, type A genotypes showed a reduction in the length of the floral cycle, the female flowers opened approximately at 12:00 and the male flowers closed before 20:00.

On the other hand, while type A genotypes showed a similar behaviour to that described for the first time by Nirody (1922), the floral cycle of type B genotypes, under our growing conditions, did not follow the normal cycle described in previous reports. Thus, flowers opened at the female stage in the evening; most of them remained open briefly the following morning overlapping with the male flowers that opened for the first time at the female stage two days earlier. A similar behaviour was described by Lesley and Bringham (1951) since the flowers did not open in the female stage under conditions where the maximum day temperatures ranged between 18 °C and 21 °C and night temperatures ranged between 7 °C and 12 °C. Under our growing conditions in April, maximum day temperatures ranged from 17.5 °C to 27.5 °C in 2005 and from 18.5 °C to 25.5 °C in 2006 (with an average of 21.6 °C and 21.4 °C respectively) whereas minimum night temperatures ranged from 9 °C to 17.5 °C in 2005 and from 11 °C to 16.5 °C in 2006 (with an average of 12.8 °C and 13.6 °C respectively). Thus, our results corroborate the higher sensibility of type B genotypes to low temperatures reported previously (Lesley and Bringham, 1951; Sedgley, 1977; Sedgley and Annels, 1981; Sedgley and Grant, 1983).

The cycle described above was observed under normal conditions in the blooming season. However, during this period, a few cloudy days resulting in lower temperatures were observed ranging from 4 days in 2005 to 7 in 2006. In these days, characterized by a decrease on day and night temperatures, the floral behaviour was altered. Thus, under cooler conditions, 'Hass' flowers showed a delay of their regular floral cycle; female flowers opened at mid-day and closed around 18:00; male flowers opened at night and remained open until mid-day (data not shown). Thus, the second flower opening took place the third day after the first opening. Under those conditions, flowers of type B cultivars usually

opened at the female stage in the evening and, while some of them closed during the night, others remained open during the morning overlapping with the male flowers that opened for the first time at the female stage three days earlier. The female flowers reopened the fourth day in the morning as male flowers. Similar observations on the alteration of the flower cycle have been reported previously (Stout, 1923; Robinson, 1931; Lesley and Bringham, 1951; Papademetriou, 1976; Sedgley, 1987).

3.3. Differences in final fruit set

Significant differences were obtained in fruit set following hand pollination with the three selected pollinizers. Thus, final fruit set was 2.8%, 7.4% and 8.4% for 'Fuerte', 'Marvel' and 'Nobel' respectively. No significant differences were found between 'Marvel' and 'Nobel' ($P = 0.558$), whereas significant differences were obtained between 'Fuerte' and 'Nobel' ($P < 0.001$) and 'Marvel' ($P = 0.001$). Taking into account these results and the results discussed above on the overlapping in the flowering cycle, it seems that 'Fuerte' is less efficient than the other two pollinizers for 'Hass' under our growing conditions. Different factors such as differences in pollen vigor or differential pollen tube growth during the progamic phase and/or differential embryo abortion at the post-zygotic level could be responsible of these differences.

4. Conclusion

Pollination is considered as a limiting factor for optimal yield production in commercial avocado orchards (Vithanage, 1990; Ish-Am and Eisikowitch, 1991). Taking into account the flowering phenology, the overlap in sexual stages and the fruit set obtained with hand-pollinated flowers in the field, an alternative to the current use of 'Fuerte' as main pollinizer in the avocado orchards in Southern Spain could be to combine some 'Fuerte' trees which will guarantee the pollination during the first two weeks of the flowering of 'Hass', with other type B genotypes such as 'Nobel' and/or 'Marvel'. This mixed plantation system of two or more genotypes would have the advantage of decreasing the possible negative effect of a lack of synchrony between two genotypes depending on the environmental conditions. A similar approach could be used in other avocado growing regions to optimize 'Hass' cross-pollination.

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