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Chilling requirements of almond seeds related to flowering time of pollen donor

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Summary

Breaking seed dormancy affects germination and growth of seedlings in *Prunus* species. Maternal tissues appear to control the seed germination process, although the effect of the pollinizer also has been described. In this work, the correlation between the pollinizer flowering times on the stratification requirements of almond seed germination was studied. Stratification requirements to germinate (number of weeks at 7°C) were determined in seeds (without endocarp) of 9 different crosses between 3 female progenitors (Marcona, Antoñeta and R1000) and 3 male progenitors (pollinizers) with different flowering dates: Achaak (early flowering), Peraleja (intermediate flowering) and S5133 (very late flowering). Results showed the influence of pollinizer flowering time on the stratification requirements of seeds germination. The mean stratification requirements of seeds followed the order of the flowering dates of pollinizers. The lack of effect of female progenitor on stratification requirements could be due to the leaching of inhibitors in the testa during disinfection and stratification of seeds.

Introduction

Seed dormancy, a physiological mechanism to delay germination and thus protect seeds of temperate fruit species from frost damage during winter, in *Prunus* has two different mechanisms: an external mechanism which inhibits seed germination controlled by the testa (maternal tissue), and another internal mechanism controlled by the embryo which also affects later growth of seedlings (Lipe and Crane 1966; Mehanna and Martin 1985; Mehanna *et al.,* 1985; Seeley *et al.,* 1998; Martínez-Gómez and Dicenta 2001). Overcoming both mechanisms is necessary for the completion of seed germination and normal growth of seedlings. Stratification in cold chambers, application of hormones and the removal of endocarp and testa are methods traditionally used to break seed dormancy in *Prunus* species including peach (Chao and Walker 1966; Diaz and Martin 1972; Zigas and Coombe 1977a; 1977b; Du Toit *et al.,* 1979; Rouskas *et al.,* 1980; Mehanna *et al.,* 1985; Seeley and Damavandy 1985; Frisby and Seeley 1993; Seeley *et al.,* 1998; Martínez-Gómez and Dicenta 2001), almond (González-Cepeda 1975; Seeley and Damavandy 1985; García-Gusano *et al.,* 2004), apricot (Seeley and Damavandy 1985), cherry (Seeley and Damavandy 1985) and *Prunus khinjuk* (Kafkas and Kaska 1998).

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Although maternal tissues (testa and endocarp) appear to control the seed germination (Chao and Walker 1966; Du Toit *et al.,* 1979; Seeley *et al.,* 1998; Martínez-Gómez and Dicenta 2001), some hypotheses have pointed to an effect of the pollinizer in seed germination in almond (Kester 1969; Grigorian, 1972; Kester *et al.,* 1977; García-Gusano *et al.*, 2004). In addition, Grigorian (1972) indicated that this influence was even more important than the influence of the seed-parent, although some of the male progenitors with late flowering time, produced seeds with low chilling requirement (e.g Cristomorto). However, studies developed in almond could indicate an absence of influence of pollinizer flowering time in the chilling requirements of seed germination (García-Gusano *et al.*, 2005).

Seeley *et al.* (1998) and Martínez-Gómez and Dicenta (2001) indicated that the genotype of the embryo did not affect the chilling requirements of peach (*P. persica* L.) seed for germination, but can affect the later seedlings growth, showing dwarf and rosette growth. Pérez-González (1990) also observed a great effect of pollinizer in seed germination in peach.

These contradictory results could be explained by the different stratification conditions used by the authors (with or without endocarp or testa, temperature) and the unsuitability of the plant material assayed, not created specifically for these studies but with breeding purposes. It is the case when both progenitors have high or low chilling requirements, and the variability within the offspring for this trait is low. Thus, in order to clarify this relationship it would be necessary to design a specific assay with a more suitable plant material crossing progenitors with a broad range (high-low) of chilling requirements.

The objective of this work was to determine in almond the relation between the pollinizer flowering time and the stratification period required for germination of their seeds in controlled crosses, just designed with this purpose.

Materials and methods

The six almond cultivars used as progenitors in this study are described in the table 1. These cultivars cover all the range of flowering times described to date in almond. Stratification requirements for germination were studied in seeds from 9 different crosses between the female progenitors Marcona (middle flowering), Antoñeta (late flowering) and R1000 (very late flowering) and three male progenitors (pollinizers) Achaak (very early flowering), Peraleja (middle flowering) and S5133 (very late flowering) (table 2). These crosses were carried out during the flowering by hand cross-pollination using a small brush, following the traditional methodology of breeding programs. The pollen was collected when flowers were closed [stage D of Fleckinger described in almond by Felipe (1977)], dehydrated 48 hours at 22°C and stored at 4°C (Martínez-Gómez *et al.,* 2000). When pollinizer had a later flowering time than the mother plant, pollen was collected the previous year and stored at -20°C (Martínez-Gómez *et al.*, 2002).

Mature fruits from the different crosses were collected during the summer in the experimental field located at Santomera (Murcia, Southeast of Spain). In these samples, the mesocarp was removed and stones stored dry at room temperature (22°C). In the

Table 1. Almond cultivars assayed. Origin, pedigree and date of flowering.

Almond cultivars	Origin	Pedigree	Flowering [a]
Achaak	Tunisia	Unknown	Very early (27)
Peraleja	Spain	Unknown	Middle (38)
Marcona	Spain	Unknown	Middle (40)
Antoñeta	Spain	Ferragnès × Tuono	Late (47)
S5133	Spain	Primorskii (Open pollination)	Very late (58)
R1000	France	Tardy Nonpareil × Tuono	Very late (66)

[a] In parentheses, the average number of Julian days (days after January 1st) for full flowering (50% flowers opened) for 3 years (2000, 2001 and 2002).

winter, seeds with endocarp were imbibed in 2% TMTD® (Tetramethilthiuram disulfide) fungicide solution for 1 hour and immersed in water for 24 hours. After this treatment endocarps, were removed and seeds were placed in plastic mesh bags in moist vermiculite (2 × 2 mm size), in a chamber at 7°C. For each crossing, the number of germinated seeds, judged by radicle protrusion of 5 mm. was recorded weekly until germination was completed. The percentage of germination was calculated as the number of germinated seeds × 100 / total number of seeds.

The time of germination of each seed was recorded as the number of weeks required for its germination. The mean germination time (MGT) was calculated as the summation of the every germination time, divided by the total number of germinated seeds in each group (progeny, male or female progenitor).

The statistical analysis was performed using the SPSS statistical packages. As the Levene test showed that variances were not homogeneous, the test of multiple comparisons T3 of Dunnet was used to determine the effect of the flowering time of the female and male progenitors on the stratification requirements to germinate their seeds.

Results

The final size of sample of each cross depended on the success of the fruit set after the controlled pollinations in the field, which use to be very variable. It ranged between 22 and 105, with an average of 71 seeds by cross. In total 637 seeds were germinated. The percentage of germination of samples was very good, ranging between 82 and 100, with an average of 89 (table 2).

Stratification requirements by progenies

Considering each female progenitor with the three pollinizers, T3 Dunnet's test identified three groups in the case of Antoñeta and R1000, with mean germination times following the order of the flowering time of these pollinizers (table 2). The later was the flowering time of the pollinizer, the later germinated the seeds (on average). In the case of Marcona, T3 Dunnet's test separated the crosses with S5133 (with higher values) from Achaak and

Table 2. Progenies and female and male progenitors studied: number of seeds stratified (N), percentage of seed germination (%), mean germination time (MGT) in weeks, range of weeks in stratification (minimum-maximum), and coefficient of variation (CV).

Female	Male	N	%	MGT [a]		Range of weeks	CV
Stratification requirements of the progenies							
Marcona	x Achaak	51	90	5.0	b	3 (4-6)	12.3
	x Peraleja	22	100	4.9	b	3 (4-6)	12.4
	x S5133	85	85	5.8	a	6 (4-9)	16.7
Antoñeta	x Achaak	50	94	4.8	c	2 (4-5)	7.9
	x Peraleja	105	98	5.3	b	4 (4-7)	10.5
	x S5133	47	91	6.0	a	6 (4-9)	19.7
R1000	x Achaak	103	83	4.7	c	4 (4-7)	14.5
	x Peraleja	92	82	5.4	b	5 (4-8)	12.9
	x S5133	82	85	5.8	a	6 (4-9)	20.5
Stratification requirements of the female progenitors							
Marcona		158	89	5.4	a	6 (4-9)	16.9
Antoñeta		202	96	5.3	a	6 (4-9)	15.1
R1000		277	83	5.2	a	6 (4-9)	18.5
Stratification requirements of the male progenitors							
Achaak		204	87	4.8	c	4 (4-7)	12.6
Peraleja		219	91	5.2	b	5 (4-8)	11.8
S5133		214	86	5.8	a	6 (4-9)	18.9

[a] Values followed by the same letter show no significant differences by T3 Dunnet multiple comparisons test ($P<0.05$).

Peraleja. Although the mean germination time followed the order of flowering time of respective pollinizers, the maximum differences between mean germination times (around one week) corresponded to more than four weeks in the flowering time of pollinizers. In general, seeds exhibited a wide range of stratification requirements within crossings (between two and six weeks). The coefficients of variation reflected clearly this variability (table 2). These ranges and coefficients of variation were higher in the case of progenies from crosses with the latest flowering pollinizer (S5133) with the three females.

Stratification requirements by female and male progenitors

Test of multiple comparisons T3 of Dunnet detected significant differences between male progenitors but did not between female progenitors for germination time (table 2).

Considering each female with all its pollinizers, T3 Dunnet's test did not detect significant differences between females for stratification requirements. On average, they presented a similar behaviour (means, range and coefficient of variation). However, considering each pollinizer with all its females, T3 Dunnet's test detected significant differences between pollinizers, the time of germination following the order of the flowering time of pollinizers (like observed by each progeny) (table 2). Again, differences of one week in seed stratification corresponded to more than four weeks in the flowering date of pollinizers.

Discussion

In our assays, the maternal effect usually related to exogenous dormancy has been reduced or eliminated. It seems that the removal of the endocarp has produced two effects. First of all we have eliminated its mechanical effect, accelerating the imbibition process and increasing the gas exchange. Secondly, the close contact of naked testa with humid vermiculite may have produced the washing out of some compounds from the testa, the hormones involved in seed dormancy included (Lipe and Crane 1966; Mehanna and Martin 1985; Martínez-Gómez and Dicenta 2001).

However, the dormancy of embryo (with equal genetic contribution of both progenitors) did not show any female influence. As far as we know, no explanation can be given to this fact. This maternal influence was indeed observed in germination of seeds with endocarp (García-Gusano *et al.,* 2004), which shows the importance of the stratification conditions on the results. These results seem to indicate that not only the female tissues control the germination (Chao and Walter 1966; Du Toit *et al.*, 1979; Seeley *et al*., 1998; Martínez-Gómez and Dicenta 2001) but also the genotype of embryo. When during stratification we remove the influence of maternal tissues (removing the endocarp and washing the testa) we facilitate the expression of pollinizer effects in the embryo.

Kester (1969) assessed that there was an important relationship between the flowering time of both progenitors and the stratification requirements of seed (without endocarp) germination, the genotype of the embryo and testa being responsible for these requirements. However, in some cases this author indicated that pollinizers with late flowering time produced seeds with low chilling requirements. Kester (1969) explained these by the incapacity of certain cultivars to transmit this characteristic.

Later, Grigorian (1972) attempted to confirm the results of Kester (1969), and indicated that the effect of the male progenitor was higher than the effect of the female progenitor on the stratification of seeds without endocarp. However, although differences between families were observed, they were not always related to pollinizer flowering date. Some seeds from pollinizers with late flowering dates had lesser stratification requirements than those from pollinizers with early flowering dates. To explain the numerous exceptions, Grigorian (1972), assessed that some cultivars could have low chilling requirements to flower, but high for seed germination.

Pérez-González (1990) also described a great effect of pollinizer in seed (with endocarp) germination in peach, indicating that genotypes with lower chilling requirements (LCR)

pollinated with pollen from genotypes with high chilling requirements (HCR) showed a delay in the germination of seeds of 16 days in comparison with the seeds from the self-pollination of the LCR. Reciprocally, HCR genotypes pollinated with pollen from LCR genotypes accelerated the germination of their seeds in around 20 days.

García-Gusano *et al.* (2005), germinating seeds without endocarp did not observe any influence of pollinizer. However, considering the low number of seeds (17) of one of the crosses involved, its mean value may not have been representative and, observing the other results, a pollinizer effect could have been showed. When seeds were germinated with endocarp, García-Gusano *et al.* (2005) did not observe any influence of pollinizer flowering time on germination. Probably, differences in the hardness or permeability of each endocarp might affect the imbibition and exchange of gases during germination, affecting time of germination.

Powell (1987), García-Gusano *et al.* (2004) and Dicenta *et al.* (2005) observed some relationship between the chilling requirements of vegetative and floral buds for sprouting and the stratification requirements of seeds for germination in peach and almond. This general relation occurs, but is not very close for two reasons. Firstly, the large differences of flowering time between cultivars are smaller in the germination time of their offspring. So, differences of one month in the flowering date correspond to only a week on average in the germination of seeds. Secondly, the variability of germination time of seeds from the same tree is usually large, until 6 weeks. So, some seeds from late flowering cultivars can germinate earlier than some seeds from early flowering cultivars.

The variability observed between and within families probably was due to the genotype of the embryo (each seed is genetically different), since the well known maternal effects of endocarp (which was removed) and testa (very likely washed out during germination) were eliminated. It seems that the higher mean values for stratification were due to the presence of seeds that need longer stratification, although in all crosses (early or late germination time) seeds with very low stratifications requirements (4 weeks) were found (table 2). These wide ranges of germination time imply an adaptive mechanism for the survival of this species against the change of environmental conditions (Leopold 1996). This variability is usually observed in other traits (flowering and ripening times, habit, vigour, fruits characteristics…) in the offspring and the more different the progenitors are the wider the variability between descendants is.

In natural germination conditions (with endocarp and tegument) the effect of pollinizer shown in this study must be overruled by the stronger maternal effect. In this sense, nature has mainly developed the maternal control of germination as a more important mechanism for the survival and germination of the seeds, which probably will grow in the same environmental conditions as the mother plant. This is logical since in nature the pollination of a plant with pollinizers of different flowering time (earlier or later) is not possible and so almond species do not need to determine the dormancy of their seeds as a function of the chilling requirements of their male progenitors. Our experimental conditions have shown the pollinizer influence on the germination of seeds, which may not be detected in natural conditions and which contribution is less important than the known maternal effect for seeds with endocarp.

In conclusion, results showed a clear relation between pollinizer flowering time and the stratification time necessary to germinate the seeds without endocarp. The lack of effect of female progenitor may be partially explained by the washing out of inhibitors from the testa during the stratification, what does not happen when the seeds are germinated with the endocarp.

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