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# Early selection for flowering time in almond breeding programs

**M. Rubio*, T. Cremades and F. Dicenta**

Plant Breeding Department, CEBAS-CSIC
P.O. Box 164, 30100 Campus Universitario de Espinardo, Murcia (Spain)
*e-mail: mrubio@cebas.csic.es

**Abstract.** Late flowering to reduce the risk of late frosts is one of the main objectives of the almond breeding programs. Breeders have to wait at least three years to know the flowering time of the seedlings, so it would be interesting to develop early selection strategies for this trait. Although some trials have been conducted correlating chilling requirements of seeds to germinate, leafing and flowering, the methodologies employed did not find a close correlation between these characteristics. In this work we studied the relationships between germination, sprouting and flowering in four families created in 2009 between very early and very late genitors that segregate well for flowering time. In winter, the seeds obtained were placed in imbibition for three days, removed the shell and stratified at 10°C in vermiculite for germination. Weekly the emergence of the root was noted as the date of germination. The plants obtained were taken to greenhouse in pots where they grew up till summer. Then they were taken to a cold room at 10°C, where weekly the number of buds sprouted was noted as the leafing time in controlled conditions. Finally, the plants were taken to the definitive plots and in 2013, 2014 and 2015 the leafing and flowering time were scored. Correlations between the requirements of seed to germinate and flowering time were no significant. Correlations between leafing time in the cold chamber and flowering time in the field several years later were intermediate, questioning the use of this correlation as early selection criterion for flowering time in almond.

**Keywords.** *Prunus dulcis* – Germination time – Leafing time – Flowering time – Early selection.

***Séléction précoce pour la date de floraison dans les programmes d'amélioration de l'amandier***

***Résumé.*** *La floraison tardive pour réduire le risque de gelées est l'un des principaux objectifs des programmes de sélection d'amandiers. Les sélectionneurs doivent attendre au moins trois ans pour connaître le temps de floraison des plants, de sorte qu'il serait intéressant de développer des stratégies de sélection précoce pour ce caractère. Bien que des essais aient été menés pour la corrélation entre les besoins en froid des graines pour la germination, la feuillaison et la floraison, les méthodologies employées n'ont pas trouvé de corrélation étroite entre ces caractéristiques. Dans ce travail, nous avons étudié les relations entre la germination, le bourgeonnement et la floraison chez quatre familles créées en 2009 entre des géniteurs très précoces et très tardifs qui se ségrègent bien pour la date de floraison. En hiver, les graines obtenues ont été placées pour imbibition pendant trois jours, dépouillées de leur coque et stratifiées à 10°C sur vermiculite pour assurer la germination. L'émergence de la racine a été notée chaque semaine en tant que date de germination. Les plantes obtenues ont été placées sous serre dans des pots où elles ont grandi jusqu'à l'été. Ensuite elles ont été emmenées dans une chambre froide à 10°C, où hebdomadairement le nombre de bourgeons ayant poussé a été noté comme temps de feuillaison en conditions contrôlées. Enfin, les plantes ont été plantées dans les parcelles définitives et, en 2013, 2014 et 2015, les temps de feuillaison et de floraison ont été évalués. Les corrélations entre les exigences de la graine pour germer et le temps de floraison n'étaient pas significatives. Les corrélations entre la date de feuillaison en chambre froide et la date de floraison aux champs quelques années plus tard ont été intermédiaires, remettant en question l'utilisation de cette corrélation comme critère de sélection précoce pour la date de floraison chez l'amandier.*

***Mots-clés.*** Prunus dulcis – *Temps de germination – Temps de feuillaison – Temps de floraison – Sélection précoce.*

## I – Introduction

Late flowering is one of the main objectives of breeding programs, since it determines the vulnerability of production to spring frosts (Dicenta *et al*., 1993; 2005, García-Gusano *et al*., 2010). Almond breeding for late flowering is a laborious task that implies the production and study of numerous seedlings. These descendants cannot be selected until the third year after planting, when the seedlings have their first flowering. For this reason, it would be interesting to use a method for early selection of late-flowering individuals, which will be the only ones planted in the orchard for a later selection for other characteristics.

Some authors studied the correlation between the flowering time and the stratification requirements of seeds for germination (Kester *et al*., 1977; Dicenta *et al*., 2005; García-Gusano *et al*., 2010) or the leafing time (Kester *et al*., 1977; Vargas and Romero, 1984; Dicenta *et al*., 2005; García-Gusano *et al*., 2010). These correlations were more or less important depending of the work but they were always affected by environmental factors that could have hidden the genetic relationship between these traits.

The objective of this work was to study the correlations between seed germination, first leafing time under controlled conditions and flowering time several years later by using a new methodology, in order to determine the accuracy of using these characteristics as criteria for early selection of late flowering in almond breeding programs.

## II – Plant material and methodology

Plant material assayed included 128 almond seeds and seedlings from 4 families created in 2009 (Table 1).

**Table 1. Mean number of days for germination (2009) and leafing (first to fifth bud sprouted) in cold room (2010) by families. Leafing time and Flowering time mean values (Julian days) for each family in the orchard in 2013, 2014 and 2015**

| | | | 2009 | 2010 | | | | | 2013 | | 2014 | | 2015 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Female | Male | N | Germination | Sprouted buds | | | | | Leafing | Flowering | Leafing | Flowering | Leafing | Flowering |
| | | | | 1st | 2nd | 3rd | 4th | 5th | | | | | | |
| Desmayo | Achaak | 31 | 38 | 87 | 100 | 100 | 102 | 108 | 28 | 18 | 31 | 27 | 41 | 33 |
| Desmayo | Tardona | 85 | 38 | 121 | 136 | 139 | 141 | 141 | 46 | 43 | 50 | 48 | 62 | 60 |
| Tardona | Desmayo | 8 | 57 | 122 | 153 | 175 | 202 | 202 | 46 | 46 | 51 | 48 | 62 | 61 |
| Tardona | D00-078 | 4 | 51 | 121 | 127 | 127 | 127 | 136 | 60 | 66 | 55 | 58 | 68 | 71 |
| TOTAL | | 128 | 42 | 110 | 122 | 125 | 125 | 127 | 42 | 36 | 46 | 43 | 58 | 54 |

During the winter 2009-2010, the nuts (with endocarp) were placed in imbibition for 3 days. Then the endocarp was removed and seeds (with tegument) were stratified at 10°C individually in cell trays covered by a permeable bag with wet vermiculite inside, without contact with the seeds. Weekly the emergence of the root (0.5 cm) was noted as the date of germination. The plants obtained in 2010 were cultivated in a greenhouse in 3 liter pots, where they grew up till summer. Then they were taken to a cold chamber at 10°C and pruned at 40 cm high removing all lateral branches. Weekly, the number of buds sprouted was noted as the leafing time in controlled conditions. Finally, the plants were taken to plots where they remained till today. In 2013, 2014 and 2015, the time for full leafing (50% of vegetative buds sprouted) and the time for full flowering (50% of floral buds opened) were recorded as Julian days (number of days after January 1st).

Pearson´s correlation coefficient was calculated between the number of days in stratification required for seed germination (2009), the time of leafing in the cool chamber (2010) for 1, 2, 3, 4 and 5 buds sprouted, the time of leafing in the field (2013, 2014 and 2015), and the time flowering in the field (2013, 2014 and 2015).

## III – Results and discussion

Table 1 shows the families studied and the mean values for the studied traits during seven years (2009-2015). We observe the maternal effect on the germination, being the female genitor the responsible for the germination, regardless the male. The families of 'Desmayo' as female were the earliest and those of Tardona the latest. In the case of leafing and flowering of seedlings, we observe a clear effect of both progenitors on the offspring, being early flowering the offspring of 'Desmayo' × 'Achaak', intermediate 'Desmayo' × 'Tardona' (direct and reciprocal) and late flowering 'Tardona' × 'D0-078'.

The weather of the three years was very different delaying the flowering time 7 days (in 2014) and 11 more in (2015), on average. In general, leafing was some 3-7 years later than flowering.

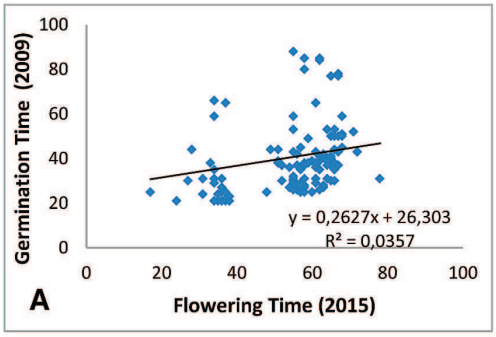
There is no correlation between the chilling requirements of seeds to germinate and the buds to sprout or flowering, any year (Table 2, Fig. 1A). In agreement with our results, Kester *et al*. (1977) and Dicenta *et al*. (2005) obtained a low correlation between the stratification requirements of seeds for germination and the time of flowering of seedlings. García-Gusano *et al*. (2010) obtained a correlation intermediate (0.5). Considering all these results, we conclude that the stratification requirement of seed is not an efficient criterion for the early selection of late-flowering cultivars.

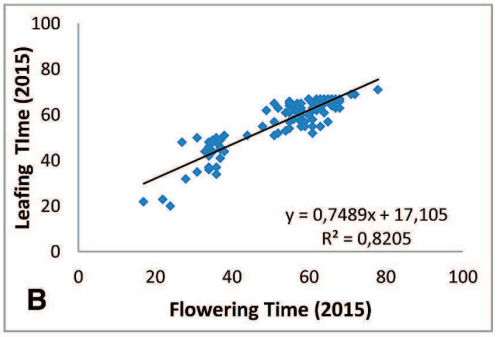
**Table 2. Pearson correlation coefficients between germination (2009), leafing time in cold chamber (2010), and leafing time and flowering time in the orchard (2013, 2014, 2015), with individual values of seedlings**

| | 2010 | 2013 | | 2014 | | 2015 | |
|---|---|---|---|---|---|---|---|
| | Leafing (chamber) | Leafing | Flowering | Leafing | Flowering | Leafing | Flowering |
| Germination | | 0.12[a] | 0.13[a] | 0.11[a] | 0.14[a] | 0.08[a] | 0.19[a] |
| Leafing (cold chamber) | 1st sprout | 0.56 | 0.59 | 0.63 | 0.57 | 0.55 | 0.60 |
| | 2nd sprout | 0.59 | 0.63 | 0.67 | 0.59 | 0.61 | 0.63 |
| | 3rd sprout | 0.57 | 0.60 | 0.59 | 0.49 | 0.56 | 0.54 |
| | 4th sprout | 0.56 | 0.59 | 0.72 | 0.66 | 0.66 | 0.68 |
| | 5th sprout | 0.55 | 0.58 | 0.61 | 0.60 | 0.57 | 0.58 |
| Leafing time (same year that flowering) | | | 0.86 | | 0.92 | | 0.91 |

All values are significant at 0.001 level except for those of germination[a] (non-significant at 0.05 level).

On the other hand, as expected, a narrow correlation (0.86-0.92) between leafing and flowering time in the same year was observed (Table 2, Fig. 1B). The relationship between chilling requirements for leafing and flowering times has been shown (Kester *et al*., 1977; Vargas and Romero, 1984; Dicenta *et al*., 2005; García-Gusano *et al*., 2010). Different correlations were observed when flowering and leafing of the same year were considered or when mean values of families were studied (Kester *et al*., 1977, Vargas and Romero 1984; Dicenta *et al*., 2005). However the only efficient tool for early selection is the individual correlation between different years. Correlations between progenitors and family means or between data of the same year are not interesting for this purpose and could be predicted because of the high heritability of this trait (Dicenta *et al*., 1993).



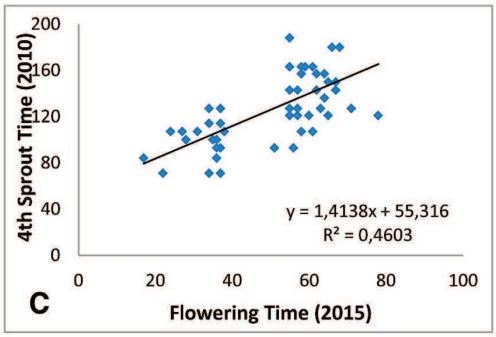




**Fig. 1. (A) Relationship between germination (number of days of stratification in 2009) and flowering time (Julian days in 2015) of seeds and the seedlings coming from them, belonging to four families of almond. (B) Relationship between leafing time (Julian days) and flowering time (Julian days) in 2015 of seedlings belonging to four families of almond. (C) Relationship between leafing (number of days in cold chamber for sprouting four buds in 2010) and flowering time (Julian days in 2015) of seedlings belonging to four families of almond.**

When individual data of first leafing in the field was compared with flowering some years later, the only useful for early selection (Dicenta *et al*., 2005), the correlation was lower. This low correlation was explained because, even if they are related, they are different traits (Sánchez Pérez *et al.*, 2012). In fact, there are almonds which flower before leafing, leaf before flowering or flower and leaf simultaneously. Furthermore, there is certain interaction genotype x environment, which brought about slight differences in the leafing and flowering times, depending on the climatic conditions of the year and the age of the tree.

Significant intermediate correlations were observed between leafing in the cold chamber in 2010 and leafing and flowering in the field in 2013, 2014 and 2015 (Table 2, Fig. 1C). These correlations varied from 0.49 (2$^{nd}$ sprouts in 2014) to 0.68 (4$^{th}$ sprouts in 2015, Table 2) between the first leafing time in cold chamber and flowering time in the field several years later, using this original methodology, that could be applied for early selection in the breeding programs, but assuming certain error.

Improvement of this interesting method could increase this correlation or perhaps we will have to assume that the first leafing (in cold chamber or field) and the first flowering some years later in the field are related traits but different enough to question their use in early selection of late almonds. Correlations between leafing time in the cold chamber and flowering time in the field several years later were intermediate, questioning the use of this correlation as early selection criterion for flowering time in almond.

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