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SUMMARY – The correlations between seed germination or leafing time and flowering time in almond [*P. dulcis* (Mill.) D.A. Webb] were studied in a population of 502 seedlings from 13 families. The aim of the study was to discover the possibilities of an early selection of those seedlings with late flowering in relation to germination of seeds or leafing time in the following year. The crosses were conducted in 1997. The seeds obtained were germinated by conventional stratification at 7°C, and the number of weeks necessary for the germination of each seed recorded. The trees were planted in 1998. Between 1999 and 2002 their leafing time was recorded, and in 2001 and 2002 also their flowering time. The results indicated that these characteristics bear a relation when considering the population globally, but with a high variability. However, within each family, the correlations between these characteristics were not important and, in most cases, not significant. In the case of germination-flowering, the lack of correlation seems to be the consequence of the germination depending mainly on the female parent. In the case of leafing-flowering, the lack of correlation was due to the positive or negative correspondence between leafing and flowering and to the genotype x environment interaction. In our opinion, to obtain seedlings with an extra-late flowering time, the selection of parents with very late flowering time is essential, with subsequent selection of the seedlings during their first flowering. An early selection regarding the leafing period could be applied with a certain degree of success when, for any reason, interested in selecting late-flowering trees coming from parents with very late and very early-flowering periods. In such cases, the high variability expected could compensate, to a certain degree, for the differences between leafing and flowering.

Key words: Almond, *Prunus dulcis*, breeding, early selection, flowering.

***RESUME –** "Sélection précoce d'amandiers à floraison tardive". Les corrélations entre la germination de la graine ou la date de débourrement et la date de floraison chez l'amandier [*P. dulcis *(Mill.) D.A. Webb] furent étudiées dans une population de 502 plantes issues de 13 familles. L'objectif de l'étude était de découvrir les possibilités d'une sélection précoce de ces plantes ayant une floraison tardive en relation avec la germination des graines ou la date de débourrement dans l'année suivante. Les croisements furent réalisés en 1997. Les graines obtenues ont germé selon une stratification conventionnelle à 7˚C, et le nombre de semaines nécessaires à la germination de chaque graine est enregistré. Les arbres furent plantés en 1998, et leur date de débourrement enregistrée entre 1999 et 2002, ainsi que leur date de floraison en 2001 et 2002. Les résultats indiquèrent que les caractéristiques ont mis en évidence une relation si l'on considère la globalité de la population, mais avec une forte variabilité. Cependant, dans chaque famille, les corrélations entre ces caractéristiques se sont avérées peu importantes et dans de nombreux cas, non signifiantes. Dans le cas de la germination-floraison, la faible corrélation semble être la conséquence du fait que la germination soit contrôlée principalement par le parent femelle. Dans le cas du débourrement-floraison, la faible corrélation serait due à une asynchronie positive ou négative entre le débourrement et la floraison, et aussi à l'interaction entre le génotype et l'environnement. Selon nous, afin d'obtenir des sélections aux dates de floraison très tardives, la sélection de parents avec une date de floraison très tardive est essentielle, avec la sélection de plantes pendant leur première floraison. Une sélection précoce prenant en compte la période de débourrement pourrait être appliquée avec un certain taux de réussite quand, quelle que soit la raison, nous voulons obtenir des sélections très tardives à partir d'un croisement entre un géniteur très précoce et un autre très tardif. Dans ce cas, la grande variabilité attendue pourrait compenser, à un certain degré, des différences entre le débourrement et la floraison.*

***Mots-clés :** Amandier,* Prunus dulcis*, amélioration végétale, sélection précoce, floraison.*

Introduction

Almond breeding for late flowering and self-compatibility is an expensive and time-consuming process, implying the production and study of large progenies, which cannot be selected until the third or the four year after planting, when the seedlings have their first flowering (Grasselly, 1972; Kester *et* *al.*,1977; Vargas and Romero, 1984; Dicenta *et al.*, 1993; Felipe, 2000). For this reason, it would be interesting to use a method for early selection of late-flowering individuals, which will be the ones planted for a later selection depending on other characteristics. Early selection is already possible in the case of self-compatibility, because of the availability of molecular markers derived from allele-specific PCR (Tamura *et al.*, 2000; Ortega and Dicenta, 2003).

In the case of the early selection for late-flowering almonds, some authors indicated the possibility of establishing a correlation between the flowering period and the stratification requirements of seeds for germination (Grigorian, 1972; Kester *et al.,*1977; Pérez-González, 1997) or the time of leafing (Kester *et al.,*1977; Vargas and Romero, 1984; Vargas *et al.,*1997; 1998).

The objective of this work was to study the correlations between seed germination or leafing time and leafing-flowering periods in 502 almond seedlings, in order to determine the accuracy of using these characteristics as criteria for early selection of late flowering in breeding programmes for this species.

Materials and methods

Plant material assayed included 502 seeds and seedlings from 13 families (Table 1). The first six families have as genitors early-flowering cultivars, and the rest of the families have at least one late-flowering cultivar as genitor.

Table 1. Pearson correlation coefficients between individual values of seeds (germination) and seedlings (leafing and flowering) within each family

Female	Male	No.	Germination		Leafing in 1999	
			Flowering in 2001	Flowering in 2002	Flowering in 2001	Flowering in 2002
'S2332'	'S4017'	72	NS	0.25*	NS	NS
'S3064'	'Peraleja'	38	NS	NS	0.48**	NS
'S3067'	'Garrigues'	15	NS	NS	NS	NS
'S3067'	'Peraleja'	17	NS	NS	0.48*	NS
'S3062'	'Garrigues'	35	NS	NS	NS	NS
'S3062'	'Peraleja'	12	NS	NS	NS	NS
'Primorskii'	'Lauranne'	41	NS	NS	NS	NS
'Primorskii'	'C2075'	14	NS	NS	NS	NS
'Lauranne'	'Desmayo'	116	0.30**	0.28*	0.28**	0.33**
'Lauranne'	'Primorskii'	50	NS	NS	0.44**	0.39**
'Lauranne'	'Marcona'	43	0.33*	NS	0.41**	0.31*
'Lauranne'	'S5133'	30	0.44*	0.37*	NS	NS
'S5133'	'Lauranne'	19	NS	NS	NS	NS
Total		502	0.41*	0.42*	0.71*	0.73*

*Significant at 5% level.
**Significant at 1% level.
NS = No significant.

Hand-pollinated crosses were performed in 1997. Nuts were harvested in summer and stratified at 7°C in darkness, for three months in winter. Seed germination was defined as the emergence of the radicle. The number of germinated seeds of each cross was recorded weekly.

Germinated seeds were sown in pots, indicating the number of weeks in stratification required for germination. Later, seedlings from these seeds were planted in the field in 1998. In 1999, 2000, 2001 and 2002 the time for full leafing (50% of vegetative buds sprouted) was recorded as the number of weeks after January 1st. In 2001 and 2002, the time for full flowering (50% of floral buds opened) was recorded as the number of days after January 1st.

Pearson's correlation coefficient was calculated between the number of weeks in stratification required for seed germination (1998), the time of the second leafing (1999), and the time of the first (2001) and the second (2002) flowering. For the statistical analysis, all data were expressed in weeks.

Results

Correlations between individual values of seeds and seedlings

Germination and flowering: No significant correlations were observed between the requirements of seeds for germination and the flowering of the seedlings, with the exception of 4 families. However, considering all the seedlings together, significant correlations were observed although they were very low (0.4) (Table 1).

Leafing and flowering: Correlations between the second leafing and the first and second flowering were variable depending on the family and the year. In most cases, they were not significant and were always below 0.5. However, considering all the seedlings together, significant correlations were observed, with a value of 0.7 (Table 1).

Correlations between means of progenitors and families

Germination and flowering: Correlations observed between the requirements of seeds for germination and the flowering of the seedlings were high (0.7) and significant at the 1% level (Table 2).

Table 2. Pearson correlation coefficients between means of parents and families for stratification requirement of seed germination, leafing time and flowering time of seedlings

	Flowering in 2001	Flowering in 2002
Germination	0.70*	0.72*
Leafing 1999	0.91*	0.93*

*All correlations were significant at the 1% level.

Leafing and flowering: Correlations between the second leafing and the first and second flowerings were also higher (0.7) than in the study of each individual, with a significance level of 1% (Table 2).

Correlations between stratification requirements of seeds for germination in 1998 and second flowering of seedlings in 2002

Table 3 shows the mean times of flowering in 2002 in each family as a function of the weeks required in stratification for seed germination in 1998 (equal or longer). These data indicate the effect of the early selection of seeds, in function of their stratification requirements in 1998, on flowering time of seedlings.

We can observe that if we had used the stratification requirements of seeds for germination as a criterion for early selection of late-flowering seedlings, the results would have been few effective. Within each family, we cannot observe any tendency of the flowering time to behave as a function of the weeks in stratification of the seeds. In some families, seeds which germinated later gave seedlings with a later flowering. However, in other cases a contrary effect was observed, for example in the case of S2332 x S4017 and S5133 x Lauranne. Considering all the families together, the increment of the flowering time was 0.9 weeks (from 6.1 weeks to 7.0 weeks) in seedlings from which seeds germinated from 4 to 11 weeks. In the case of 12 weeks in stratification, mean flowering time was 6.1 weeks, although the number of seedlings was very low.

Table 3. Mean flowering time in 2002 for each family, as a function of the weeks required for seed germination in 1998 (equal to or longer than that indicated)

Female	Male	Weeks in stratification								
		4	5	6	7	8	9	10	11	12
'S2332'	'S4017'	5.5	5.5	4.9	-	-	-	-	-	-
'S3064'	'Peraleja'	5.2	5.2	5.2	5.2	5.2	5.1	5.2	5.4	5.2
'S3067'	'Garrigues'	5.2	5.2	-	-	-	-	-	-	-
'S3067'	'Peraleja'	5.4	5.5	-	-	-	-	-	-	-
'S3062'	'Garrigues'	5.2	5.5	5.4	5.4	5.4	-	-	-	-
'S3062'	'Peraleja'	5.2	5.2	5.6	5.6	5.4	-	-	-	-
'Primorskii'	'Lauranne'	7.6	7.6	7.5	7.5	7.6	7.6	7.4	7.9	7.9
'Primorskii'	'C2075'	7.2	7.2	7.4	7.4	7.2	7.2	7.7	-	-
'Lauranne'	'Desmayo'	5.5	5.5	5.5	5.5	5.6	5.8	5.6	6.3	-
'Lauranne'	'Primorskii'	7.5	7.5	7.5	7.5	7.5	7.4	7.5	7.5	-
'Lauranne'	'Marcona'	6.2	6.2	6.1	6.2	6.3	6.2	6.6	6.6	-
'Lauranne'	'S5133'	8.0	8.0	8.0	8.1	8.0	8.2	8.4	-	-
'S5133'	'Lauranne'	7.8	7.7	7.8	7.8	7.6	7.6	7.2	7.7	-
Total		6.1	6.2	6.5	6.6	6.8	7.0	7.1	7.0	6.1

Shaded cells ( ) indicate the range of germination for each family.

Correlations between second leafing (1999) and second flowering (2002) of seedlings

Table 4 shows the mean time of flowering in 2002, in each family, as a function of the time of leafing of seedlings in 1999 (equal or later). These data indicate the effect of the early selection of seedlings for late flowering in function of their time of leafing in 1999.

Table 4. Mean flowering time in 2002 for each family, as a function of leafing time in 1999 (equal to or later than that indicated)

Female	Male	Weeks for leafing						
		5	6	7	8	9	10	11
'S2332'	'S4017'	5.5	5.5	5.5	5.6	-	-	-
'S3064'	'Peraleja'	5.2	5.2	5.4	5.6	-	-	-
'S3067'	'Garrigues'	5.2	5.3	5.6	-	-	-	-
'S3067'	'Peraleja'	5.4	5.4	6.0	-	-	-	-
'S3062'	'Garrigues'	5.2	5.3	5.2	5.1	-	-	-
'S3062'	'Peraleja'	5.2	5.2	5.5	-	-	-	-
'Primorskii'	'Lauranne'	7.6	7.6	7.6	7.6	7.6	7.5	-
'Primorskii'	'C2075'	7.2	7.2	7.2	7.2	7.2	7.2	6.5
'Lauranne'	'Desmayo'	5.5	5.5	5.6	5.7	6.4	5.7	-
'Lauranne'	'Primorskii'	7.5	7.5	7.5	7.5	7.5	7.8	7.9
'Lauranne'	'Marcona'	6.2	6.2	6.3	6.5	-	-	-
'Lauranne'	'S5133'	8.0	8.0	8.0	8.0	8.1	8.3	-
'S5133'	'Lauranne'	7.8	7.8	7.8	7.8	7.7	7.6	-
Total		6.1	6.1	6.6	7.0	7.6	7.6	7.2

Shaded cells ( ) indicate the range of leafing for each family.

In general, we cannot observe an important delay of the flowering time of each family in function of the leafing time observed in 1999. If, in each family, we had selected late-leafing seedlings, we would have delayed the flowering time by 0.6 weeks on average. In some families, however, this delay was null or even negative, as in the case of S2332 x Garrigues, Primorskii x Lauranne, Primorskii x C2075, and S5133 x Lauranne. On the other hand, considering all the families together, the increment of the flowering time after selecting late-leafing seedlings was 1.5 weeks. In the case of week 11, the number of seedlings studied was very low and not representative.

Discussion

Individual correlations are the only efficient tool for early selection. Correlations between progenitors and family means are not interesting for this purpose and could be predicted because of the high heritability of this trait (Dicenta *et al.*, 1993).

Correlation between germination and flowering

Correlations between germination and flowering time were, in general, not significant within each family, which could indicate the lack of a strong relationship between the stratification period required for seed germination and the time of flowering of the seedlings obtained from these seeds. The significant (but low) correlation observed with individual data of all the families could be due to the effect of the female genitor. On average, female genitors with a late-flowering time produce seeds with larger requirements of stratification, although with a high variability, and the descendants will leaf and flower later. This fact produced some degree of correlation when we considered all the families, but not in the individual analyses within each family.

In agreement with our results, Kester *et al.* (1977) obtained a low correlation (0.129) between the stratification requirements of seeds for germination and the time of flowering of seedlings. This correlation was higher (0.854) in the case of the analysis of the mean values of the progenitors and families. Considering all these results, we conclude that the stratification requirements of seeds is not an efficient criterion for the early selection of late-flowering cultivars.

Correlations between leafing and flowering times

The correlation between leafing and flowering time was variable, depending on the families, and was not significant in most cases. In the case of families with genitors having larger differences in flowering time (Lauranne x Desmayo, for example), correlations were always significant, although low. However, when we consider all the individuals (when the flowering range is larger), correlations were higher and significant.

The lack of a high correlation between the leafing of the second year in the field and the first or second flowering, within each family, could be mainly due to three factors. First of all, they are related but they are not the same trait. Secondly, the different order of leafing and flowering in almond. There are almonds which flower before leafing, leaf before flowering or flower and leaf simultaneously. These differences, depending on the genotype and the environment, could be up to two weeks either way. This fact could produce the selection of two trees with the same leafing time but with their flowering time differing up to 4 weeks. Finally, the 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.

Our results seem to indicate that there is a certain correlation between leafing and flowering although, it is not enough to use as a strategy for early selection in the case of crosses with progenitors of similar flowering time. When the two progenitors have very different flowering times, and so the variability expected within the family is large, we could use the early selection but we have to assume an important error. From the practical point of view, when, in our studied population, we simulated the early selection, we did not obtain an appreciable delay in the time of flowering, and the effect was due only to the differences between families. For example, the selection of individuals with time of leafing greater than 9 weeks would have produced the elimination of all the individuals of the early-flowering families and only a slight increase of the mean time of flowering of the later-flowering families.

Kester *et al.* (1977) observed a low correlation (0.34) between leafing and flowering of the same year in four-year-old descendants and questioned the utility of the use of leafing time as an efficient strategy of early selection for late flowering. Vargas and Romero (1984) included the leafing time as a criterion for early selection of late-flowering seedlings, finding an intermediate correlation (0.56) between these traits the same year, in the study of 602 descendants. In both works, flowering and leafing times of seedlings of the same year were correlated, but authors did not correlate the second leafing and the first flowering time, the only correlation useful for early selection. Later, Vargas *et al.* (1997) and Vargas *et al.* (1998) used the leafing of one-year-old seedlings as a criterion of early selection for late flowering of 5000 descendants obtained between 1992 and 1996, although no data regarding the efficiency of this strategy was shown.

From our point of view, while we await molecular markers for late-flowering selection, it would be more efficient to use progenitors of as late flowering time as possible, selecting the latest-flowering descendants at their first flowering. The early selection of late-leafing seedlings could be applied as a strategy for the selection of late-flowering descendants in the case of crosses between genitors with very widely differing flowering times. In this case, the wide variability expected could reduce, to some degree, the differences between leafing and flowering.

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