## Genetic variability and pollen effect on the transmission of the chemical components of the almond kernel

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### Abstract

The chemical components of the almond kernel are traits contributing to almond quality and must be considered in breeding programs searching for high-quality almonds. Thus, total protein, oil content, fatty acid composition and tocopherol content were determined in 200 genotypes from four progenies from the almond (*Prunus amygdalus* Batsch) breeding program of *Centro de Investigación y Tecnología Agroalimentaria de Aragón* (CITA), Spain. All components showed significant differences among genotypes, as well also among progenies, showing a significant effect of the pollen parent in the average composition of each family. The protein content ranged between 8.4 and 24.7%, and the oil content between 37 and 67.5% of dry matter (DM). The major fatty acid was oleic, followed by linoleic. Alphatocopherol was predominant among the different tocopherol homologues ( $\alpha$ ,  $\delta$ , and  $\gamma$ ). Significant correlations were found among some components, such as total oil, oleic acid, and  $\gamma$ - and  $\delta$ -tocopherol, but not with  $\alpha$ -tocopherol. Heritability estimates were high for oil content and  $\gamma$ -tocopherol, medium for linoleic acid and  $\alpha$ -tocopherol, and low for the rest of components. These results remark the importance of the genetic background of an individual for the quality profile of its kernels and the significant and positive effect of the pollen on the transmission of most chemical components of the almond kernel.

Additional key words: fatty acids; heritability; kernel quality; oil content; protein; *Prunus amygalus* Batsch; to-copherol.

#### Resumen

## Variabilidad genética y efecto del polen en la transmisión de los componentes químicos de la pepita del almendro

Los componentes químicos de la pepita del almendro son productos que contribuyen a la calidad del almendro y deben considerarse en los programas de mejora para almendras de mayor calidad. Por ello, se determinaron los contenidos en proteína total, en aceite y en tocoferol, así como la composición en ácidos grasos, en 200 genotipos de cuatro familias del programa de mejora genética del almendro (*Prunus amygdalus* Batsch) del Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), España. Todos los componentes mostraron diferencias significativas entre genotipos, así como entre familias, resultando en un efecto significativo del parental masculino en la media de las composiciones en cada familia. El contenido en proteína osciló entre 8,4 y 24,7%, y en aceite entre 37 y 67.5% del peso seco (DM). El ácido graso más abundante fue el oleico, seguido por el linoleico. El  $\alpha$ -tocoferol fue el homólogo predominante entre los tres mayoritarios ( $\alpha$ ,  $\delta$ , y  $\gamma$ ). Se encontraron correlaciones significativas entre algunos componentes, como el aceite total, el ácido oleico y los  $\gamma$ - y  $\delta$ -tocoferol, pero no con el  $\alpha$ -tocoferol. La heredabilidad fue elevada para el contenido en aceite y en  $\gamma$ -tocoferol, media para el ácido linoleico y el  $\alpha$ -tocoferol, y baja para el resto de los componentes. Estos resultados resaltan la importancia de la base genética de un individuo para el perfil de calidad de sus pepitas, así como el efecto positivo del polen en la transmisión de la mayoría de los componentes químicos de la pepita del almendro.

**Palabras clave adicionales**: ácidos grasos; calidad de la pepita; contenido en aceite; heredabilidad; proteína; *Prunus amygalus* Batsch; tocoferol.

Abbreviations used: CITA (Centro de Investigación y Tecnología Agroalimentaria de Aragón); DM (dry matter); FAME (fatty acid methyl esters); HPLC (high performance liquid chromatography); PUFA (polyunsaturated fatty acids).

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## Introduction

Almond is one of the mostly consumed nuts in the world. Its beneficial effects on human health have been widely reported, especially in relation to the blood lipid profile and the risk of heart diseases (Chen et al., 2006). The nutritional value of the almond kernel stems mainly from its lipid content, with a higher level of monounsaturated and polyunsaturated fatty acids (PUFA) than of saturated fatty acids. This fat profile is highly recommended in human nutrition. Among the fatty acids, oleic and linoleic acids represent over 90% of the total lipid content. The predominance of unsaturated fatty acids in almond is a very important fact from the nutritional and healthy viewpoints, since these fatty acids do not contribute to the synthesis of cholesterol in the human body (Chen et al., 2006). Therefore almond, along with other nuts, plays an important role in the human diet (Socias i Company et al., 2008).

Most vegetable oils contain different levels of tocopherols, natural anti-oxidants helping to maintain quality by increasing the resistance to oxidation and decreasing the flavour deterioration resulting in rancidity. The most interesting and abundant tocopherol is  $\alpha$ -tocopherol, one of the most important components of vitamin E in human nutrition. Other homologues such as  $\gamma$ -tocopherol protect the cell membranes from free radical damage. The genotype, the location and the environmental conditions may influence the chemical composition of the almond kernel (Romojaro et al., 1988; Abdallah et al., 1998; Kodad et al., 2006). The year effect, however, did not show interaction with genotype (Kodad et al., 2006), thus allowing the comparison between genotypes in a single year. In addition, year and sample repeatabilities are very high. Some components, such as oil content, depend mainly on the genotype and to a much lesser degree on the environmental conditions. Under the climatic conditions of CITA, the year effect was not significant for oil content and for the percentages of palmitic and stearic acids (Kodad and Socias i Company, 2008a; Kodad et al., 2010), confirming that the year stability of each fatty acid depends on the specific characteristics of the genotype (Abdallah et al., 1998).

The total phenotypic variance in a population may be due to genetic and environmental factors. Heritability analyses estimate the relative contributions by genetic and non-genetic factors to this variance. The heritability of the major agronomic traits has been estimated in many fruit and nut species, including almond (Kester *et al.*, 1977). The heritability of some additional physical traits of the almond fruits has been also determined (Arteaga and Socias i Company, 2002), but there is no information on the heritability of the chemical components of the almond kernel. It has been suggested, however, that most quality traits in almond are quantitatively inherited and subjected to polygenic control (Grasselly, 1972).

The kernel chemical components have been considered as one of the selection criteria in almond breeding programs, widening the possibilities of improving almond cultivars. Correlations between traits are highly interesting to determine whether selection for one trait could have an effect over another. The lack of significant correlations between the chemical components and the physical traits of the almond kernel (Kodad and Socias i Company, 2006), points out to the possibility of attempting the improvement of the kernel chemical composition in almond breeding without loosing the advances obtained in the kernel physical quality.

The knowledge of the genetic parameters is very useful in order to make predictions of genetic progress among seedlings in a breeding program (Falconer, 1970). Heritability also provides information for examining major changes in the amount and the nature of genetic variability through generations (Hansche *et al.*, 1972), influencing also the efficiency of selection and the cost of handling a large number of seedlings (Socias i Company, 1998). However, the chemical analyses present greater problems for their introduction in the breeding programs because some determinations are made with different analytical procedures and, as a consequence, are difficult to compare.

From the qualitative point of view, any new valuable almond genotype would be required to produce kernels with a high amount of several components, such as oleic oil, that of higher quality from the nutritional and healthy point of view, as well as of  $\alpha$ -tocopherol, because of its antioxidant activity (vitamin E), and of  $\gamma$ -tocopherol, because of its cardio-protective effect of cell membranes from free radical damage. Thus, our objective was the determination of the effect of different pollen sources on the transmission of the chemical kernel components in several breeding progenies, as well as their heritability by variance components, and the correlations between the different compounds and their genetic variability, in order to ascertain the possibility of including these compounds in a breeding program with a significant success.

## Material and methods

#### Plant material and sampling

The study included a total of 200 individuals belonging to four families from the almond breeding program of the CITA, located at 41° 38' N and 0° 53' W, at 220 m above sea level. The seedlings come from crosses between two Spanish local selections ('Bertina' and 'Vivot') and four previous releases from the same breeding program ('Aylés', 'Blanquerna', 'Felisia', and 'Moncayo'). These parents were selected because of their interesting characteristics, such as fruit quality and late blooming, as described in Felipe (2000). The individual crosses are 'Vivot' × 'Blanquerna' with 81 seedlings, 'Felisia' × 'Aylés' with 25, 'Felisia' × 'Bertina' with 62, and 'Felisia'  $\times$  'Moncayo' with 32. The trees are grown as living plants in nursery rows, maintained using standard management practices. The pollen parents were also included for comparison of the pollen effect.

The almond samples were collected from the 2007 crop. Approximately 50 mature fruits were randomly collected from each genotype. The fruit was considered mature when the mesocarp was fully dry and split along the fruit suture and the peduncle was near to complete abscission (Felipe, 2000). After discarding the mesocarp, the fruits were left at room temperature for 2-3 weeks. After reaching constant weight, 20-30 fruits from each genotype were cracked and seed coats were removed by pouring in warm water (100°C) during 5 min. Blanched kernels were again dried until constant weight and then ground in an electrical grinder to obtain fine flour. This flour was stored in a freezer at 4°C until further analysis.

#### **Oil extraction**

Total oil content was determined with a 3 g sample in a Soxtec Avanti 2055 fat extractor (Foss Tecator, Höganäs, Sweden) using 70 mL of petroleum ether as solvent (boiling point range 40-60°C) and maintaining the heat source at 135°C for a period of 2 h because previous checks showed that extraction is then practically completed, with no differences with an extraction period of 4 h (Kodad and Socias i Company, 2008b). Ether extracts containing almond kernel oil were subjected to vacuum evaporation at  $100 \pm 1$ °C to remove ether during 15 min. Subsequently, the ether extracts were tempered in a vacuum dessicator and weighed gravimetrically to determine the oil content. Extracted oil was added to 10  $\mu$ L of butylated hydroxytoluene (BHT) methanolic solution as an antioxidant agent and kept in an amber vial at  $-20^{\circ}$ C in the freezer until required for analysis. The oil extraction was duplicated using 30 fruits of each genotype. The average values are reported as differences in weight of the dried kernel sample before and after extraction.

#### Determination of fatty acid composition

The relative percentage of the different fatty acids in the oil was determined by capillary gas chromatography of the fatty acid methyl esters (FAMEs). These FAMEs were prepared by trans-etherification with KOH according to the official method UNE-EN ISO 5509:2000 (ISO, 2000). The FAMEs were separated using a gas chromatograph HP 6890 and afterwards detected using a flame ionization detector, equipped with a capillary column (HP-Innowax 30 m×0.25 mm i.d.) and 0.25 µm film thickness (Agilent Technologies, Waldbronn, Germany). The carrier gas was helium and the flow rate was 1 mL min<sup>-1</sup>. The temperature of the injector and detector was maintained at 220 and 275°C respectively. The initial column temperature was 100°C for 3 min. The oven temperature was gradually increased from 100 to 240°C, as follows, from 100 to 150°C at 2.5°C min<sup>-1</sup> ramp rate, from 150 to 200°C at 3°C min<sup>-1</sup> ramp rate, and from 200 to 240°C at 13°C min<sup>-1</sup> ramp rate and it was maintained at 240°C for 4 min. Injection volume was 1.0 µL. FAME identification was based on retention times as compared with those of the standard FAME mixture (Sigma-Aldrich, Madrid, Spain). The fatty acid determination was duplicated using 30 fruits of each genotype. The average values are expressed as percentage of each fatty acid in the total oil amount.

#### **Determination of tocopherol content**

The tocopherol content was determined according to a modification of a method already described (López Ortiz *et al.*, 2008). The individual tocopherol isomers were analyzed using a reversed phase by high performance liquid chromatography (HPLC), model 360 (Kontron, Eching, Germany) equipped with a double piston pump and self-sampler. The column used was Luna 100RP-18 of 200 mm  $\times$  4.6 mm i.d., 5 mm (Phenomenex, Torrance, CA, USA) kept at 30°C. Analytical separation of tocopherol homologues was achieved with an isocratic elution of acetonitrile:methanol (30:70). Total run time and flow rate were 19 min and 1.2 mL min<sup>-1</sup>, respectively. Samples of 0.3 g of almond oil were dissolved in 2 mL of 1-propanol by shaking at air temperature for 30 s. After 10 min of rest in dark, 20  $\mu$ L of the propanolic extract were injected in the HPLC. The amount of  $\delta$  and  $\gamma$ -tocopherol was detected with a FLD SFM25 fluorescence detector (Kontron), set at 295 nm excitation wavelength and 325 nm emission wavelength. The DAD 440 diode array detector (Kontron) at 295 nm wavelength was used to detect  $\alpha$ -tocopherol.

Identification of chromatographic peaks was based on retention times by comparison with known standards (Sigma-Aldrich). The tocopherol content determination was duplicated using 30 fruits of each genotype, reporting the average values.

#### Determination of total protein content

The protein fraction was determined through the total N content obtained by the Dumas method and applying a conversion factor as shown: % Protein = Kc \* % total Nitrogen (Kc = 6.25).

A sample of 0.2 g of almond flour was weighed and introduced into the analyser LECO FP-528 Protein/ Nitrogen Analyzer (LECO Corporation, Saint Joseph, MI, USA). The results were read and interpreted with the software CPU-CAR-02. The sample was burned at  $850^{\circ}$ C, and in the incineration CO<sub>2</sub>, H<sub>2</sub>O, N<sub>2</sub> and NO<sub>x</sub> were generated. The gas was passed through hot copper to remove oxygen and then conducted through two filters. Finally, the molecular nitrogen with helium was measured in a cell differential thermoconductivity. The results were expressed as percentage of nitrogen by weight. The protein content determination was duplicated using 30 fruits of each genotype, reporting the average values.

#### Data analysis

The experimental design was completely randomized with two replications. Analysis of variance was performed to detect differences among the families and to estimate the variance components by GLM using SAS<sup>®</sup> software (SAS Institute Inc., Cary, NC). Significant differences were recorded at p < 0.05. In addition, the analyses of Pearson correlation were done to conclude relationships among parameters using SAS<sup>®</sup> software (SAS Institute Inc.).

Estimates of heritability were calculated based on family means. Estimates of genetic variance components from progeny analyses were used to calculate  $h_n^2$  as follows:

$$h_n^2 = \sigma_{gF}^2 / (\sigma_{gF}^2 + \sigma^2 / r + \sigma_w^2 / rn)$$

where  $\sigma_{gF}^2$  is variance component (additive variance component and dominance (non-additive) variance component) due to family;  $\sigma^2$  and  $\sigma_w^2$  are replication by FS-family interaction (error) and variance within FS-families, respectively; r and n show the number of replications and the number of individual plants per family, respectively (Falconer, 1970).

#### **Results and discussion**

# Genetic variability and phenotypic correlations

The frequency distributions, the ranges of variability and the phenotypic correlations for the chemical components of the almond kernel (Fig. 1, and Tables 1 and 2) showed a similar variability to that described in other studies, although the ranges diverge for some components.

As expected, our results showed a wide range of variation, as found in other studies. The content of protein ranged between 8.4 and 24.7%, similar, although with lower values, that that already obtained in the CITA for other almond genotypes of 11.8-31.8% (Kodad, 2006). This range, however, was wider than that obtained with a lower amount of genotypes of 23.0-24.0% (Barbera et al., 1994). However, not always comparisons are possible, as it happens with the protein content, which was found to be lower than that obtained in other studies. Nevertheless, protein is being determined by different analytical methods, as well are different the conversion ratios from nitrogen to protein applied. In addition, differences may be due to the different environmental conditions and the different plant material (Barbera et al., 1994), as confirmed by the fact that the coefficient of variation between years showed that protein has a large genetic variation (Kodad, 2006). The range of variability of 3.1% agreed with other results (Kodad, 2006).



**Figure 1.** Frequency distributions for the chemical components of the almond kernel in the four families 'Felisia' × 'Aylés' (1a), 'Felisia' × 'Bertina' (1b), 'Felisia' × 'Moncayo' (1c), and 'Vivot' × 'Blanquerna' (1d). Values for parents are indicated by arrows: F, 'Felisia'; A, 'Aylés'; B, 'Bertina'; M, 'Moncayo'; V, 'Vivot'; Bq, 'Blanquerna'.

The total oil content ranged from 37.0 to 67.5%, confirming that the lipid fraction is the major component of the almond kernel. These results agree with those obtained in other European cultivars (Kodad, 2006). The content of the two most important fatty acids, oleic and linoleic, varied among genotypes, with a range of 57.4-78.4% for oleic acid and 13.4-32.2% for linoleic acid. These two fatty acids represent more than 90% of the total oil content, whereas palmitic and stearic acids represent less that 10%, as in other almond cultivars (Abdallah *et al.*, 1998). The highest range of variation among all fatty acids was observed for stearic acid with 7.8%, whereas for oil content the range of variation was 7.0%.

The most abundant antioxidant found in almond was  $\alpha$ -tocopherol, which ranged between 69.0 and 612.1 mg kg<sup>-1</sup> of kernel oil, followed by  $\gamma$ - and  $\delta$ -tocopherol. The values obtained for  $\alpha$ -tocopherol were higher than those previously reported (Kodad, 2006), probably due to higher temperatures during the growing season (Kamal-Eldin and Anderson, 1997; Kodad *et al.*, 2006) or to differences in the analytical methods employed. These differences have also been reported in other crops such as *Brassica napus* or olive (Goffman and Becker, 2001). The wider range of variation was observed for  $\gamma$ -tocopherol (8.7%), and the lowest for  $\delta$ -tocopherol (3.1%).

Regarding phenotypic correlations, a negative correlation (Table 2) was found between the oil and the total

	Protein	Oil	C16:0	C18:0	C18:1	C18:2	α-tocopherol	γ-tocopherol	δ-tocopherol
Parent									
Aylés	26.7	56.2	5.9	2.9	70.1	20.1	484.7	13.1	0.4
Bertina	24.3	58.3	6.3	2.8	69.5	20.3	375.1	12.7	0.5
Blanquerna	11.9	63.7	5.5	3.6	73.3	17.5	305.5	12.7	0.8
Felisia	12.5	50.7	6.7	2.1	69.3	20.0	389.0	25.6	1.7
Moncayo	18.7	56.5	5.8	2.2	74.5	16.7	367.3	27.8	1.2
Vivot	15.7	56.8	7.0	2.2	63.2	28.6	221.5	7.1	0.2
Family									
Felisia×Aylés	11.3ª	62.3 <sup>d</sup>	5.8ª	2.7°	71.8°	18.5ª	402.8 <sup>b</sup>	29.1°	1.6°
Felisia×Bertina	12.5 <sup>b</sup>	58.2 <sup>b</sup>	5.9ª	2.1ª	70.3 <sup>b</sup>	19.8 <sup>b</sup>	389.9 <sup>b</sup>	23.4 <sup>b</sup>	1.1 <sup>b</sup>
Felisia×Moncayo	11.3ª	60.4°	5.8ª	2.2ª	72.2°	18.6ª	387.3 <sup>b</sup>	32.0 <sup>d</sup>	1.8 <sup>d</sup>
Vivot×Blanquerna	18.8°	55.8ª	6.4 <sup>b</sup>	2.6 <sup>b</sup>	65.1ª	24.6°	353.3ª	6.7ª	0.2ª
Overall mean ± SD Minimum value Maximum value	$13.5 \pm 3.1$ 8.40 24.70	$59.1 \pm 5.7$ 37.00 67.50	$6.0 \pm 0.4$ 4.91 7.56	$2.4 \pm 0.5$ 1.41 4.50	$69.8 \pm 5.5$ 57.43 78.47	$20.4 \pm 3.9 \\ 13.36 \\ 32.16$	$383.3 \pm 83.2$ 68.96 612.05	$22.8 \pm 12.6 \\ 1.97 \\ 62.24$	$1.2 \pm 0.8$ 0.01 5.50

Table 1. Content of protein, oil, major fatty acids and tocopherol homologues in four almond progenies

The protein and oil contents are given as a percentage of kernel dry weight; the fatty acid composition is given as a percentage of the total oil content; the tocopherol homologues are given as mg kg<sup>-1</sup> oil. Values followed by different letters in the same column are significantly different at p < 0.05.

protein content ( $R^2 = -0.47$ ), as expected for the two main components of the almond kernel (Kodad, 2006). This interdependence can be explained biochemically, because both fractions are formed during the ripening process from carbohydrates, which are abundant in the early stages of seed development but that decrease all over the ripening process (Saura-Calixto *et al.*, 1988). A positive correlation of 0.38 was found between oleic acid and oil content. Similar results have been observed in other species, such as olive (León *et al.*, 2004). The correlations between total oil content and  $\gamma$ - and  $\delta$ -tocopherol were significant and positive, according with other species such as rapeseed (Goffman and Becker, 2001). The positive correlation between oleic acid and oil content suggests that in a breeding program both parameters can be selected together, because when selecting for high oil content, the content of oleic acid may be additionally increased, improving the nutritional value of the kernel and increasing the stability against rancidity. With an increase of the oil content, the amount of  $\delta$ - and  $\gamma$ -tocopherol will also be increased, but not that of  $\alpha$ -tocopherol, which is the most important and abundant tocopherol protecting the seed against oxidation (Zacheo *et al.*, 2000). A negative correlation was found between the contents of oleic and linoleic acids ( $R^2 = -0.63$ ), as expected for the two main components of the lipid fraction. The correlations between the three tocopherol homologues were positive and significant as already observed in almond (Abdallah *et al.*, 1998; Kodad, 2006). Linoleic acid, a

<b>Table 2.</b> Correlation coefficients between the chemical components of the almond ker
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Component	C16:0	C18:1	C18:2	α-tocopherol	γ-tocopherol	δ-tocopherol	Protein
Oil	-0.24**	0.38**	-0.15**	ns	0.35**	0.30**	-0.47**
C16:0		-0.35**	0.38**	-0.21**	-0.49**	-0.12**	0.32**
C18:0		-0.20**	0.31**	-0.21**	-0.16**	-0.21*	ns
C18:1			-0.63**	ns	0.48**	0.41**	-0.52**
C18:2				-0.23**	-0.27**	-0.23**	ns
α-tocopherol					0.33**	0.50**	-0.16**
γ-tocopherol						0.85**	-0.67**
$\dot{\delta}$ -tocopherol							-0.59**

Correlations non significant (ns) or significant at p < 0.05 (\*) or p < 0.01 (\*\*).

PUFA, contributes significantly to the deterioration of food quality in the presence of catalysts of the oxidation reaction such as enzymes, light and moisture (Davies *et al.*, 1987). So, if the concentration of linoleic acid decreases, food quality may increase. In other goods with high fat content, such as peanut and sunflower, the presence of a high concentration of linoleic acid produces oil instability if subjected to high temperatures during the industrial process (Motilva *et al.*, 2001).

#### Pollen effect and heritability estimation

The distribution of the different values in three families with a common parent (Fig. 1) allows analyzing the pollen effect on the transmission of the traits to the progenies. The most significant differences were found among two tocopherol homologues as shown in Table 1. The cross 'Felisia'× 'Moncayo' induced the highest content of  $\delta$ - and  $\gamma$ -tocopherol and significant differences were found among the two other families. In relation to  $\alpha$ -tocopherol, no significant differences were found between the three families obtained from 'Felisia'. The family 'Felisia' × 'Bertina' showed the highest values of linoleic acid and of protein content. The highest content on oleic acid was recorded in the families 'Felisia' × 'Aylés' and 'Felisia' × 'Moncayo', whereas no significant differences were found between them. The same trend was also found in the family 'Felisia'  $\times$ 'Aylés', which showed the highest contents for oil content and stearic acid.

The comparison of the average data of each family with the values of the pollen parent for each component showed that in most components the pollen effect is manifested in the same relationship than in the parents. Thus, the average values for the 'Felisia' × 'Aylés' family for stearic acid and  $\alpha$ -tocopherol are the highest, as they are also the highest in 'Aylés'. The same trend was observed for palmitic and linoleic acids in the family 'Felisia' × 'Bertina', and for oleic acid and  $\delta$ - and  $\gamma$ -tocopherol in the family 'Felisia' × 'Moncayo'. However, for the total contents of protein and oil no pollen effect was observed. When the fourth family, 'Vivot' × 'Blanquerna', was also included, it showed the highest values for linoleic and palmitic acids and for protein content, but also the lowest values for  $\delta$ - and  $\gamma$ -tocopherol. Hence, the contents of the different chemical components of the almond kernel mostly depend on the genotype and may be considered as varietal traits (Kodad et al., 2009). As the traits analysed are quantitative, the differences would be due to the accumulation of additive genes in the genotype.

In our case, as the effect of pollen is positive, there will be a good transmission from parents to progenies. The value of the chemical components of some seedlings was higher than in the parents, so there is an improvement in food quality, creating new material for future almond breeding programs. Some seedlings with a 500-550 mg kg<sup>-1</sup> of kernel oil for  $\alpha$ -tocopherol or 28-30% of linoleic acid, much higher than the value of the parents, have been observed (Fig. 1). The  $\alpha$ -tocopherol content has been successfully increased without increasing or decreasing the content of the other tocopherol homologues through classical breeding methods in rapeseed and maize (Goffman and Becker, 2001).

The heritability estimates for different traits depend on the variation between the genetic and the environmental effects (Souza *et al.*, 1998). A trait with high heritability indicates an additive gene action (Yao and Mehlenbacher, 2000) and will be less influenced by the environment. The selection in traits with high heritability will be very effective, thus being easier to improve. In almond the year variation tends to affect the chemical composition of the almond, but all components are affected equally within a year (Kodad *et al.*, 2006). Thus, the year effect must not affect the estimation of the heritability of these chemical compounds, taking into account that heritability estimates vary mostly with the nature of the test population (Marame *et al.*, 2009).

The heritability estimates may be ranged as high (50-100%), medium (25-50%) or low (0-25%) (Falconer, 1970). Oil content and the amount of  $\gamma$ -tocopherol showed the higher heritability estimates, with  $h^2 = 57.0\%$ and  $h^2 = 60.0\%$ , respectively. This high heritability indicates that the additive gene action predominates for these traits and that the potential effectiveness of selecting for them is very high. When heritability estimates are moderate, as in the case of linoleic acid  $(h^2 = 25.0\%)$  and  $\alpha$ -tocopherol  $(h^2 = 20.5\%)$ , selection of parents based on their phenotypes should also be effective, as it happens when heritability is high (Hansche et al., 1972). In contrast, the characters with low heritability, such as  $\delta$ -tocopherol (h<sup>2</sup> = 11.0%), oleic acid (h<sup>2</sup> = 10.5%), palmitic acid ( $h^2 = 15.3\%$ ), stearic acid ( $h^2 =$ 11.0%) and protein content ( $h^2 = 12.1\%$ ), would be more difficult to improve because they will be mostly dependant on the environment.

These results suggest a significant and positive effect of the pollen on the transmission of most chemical components of the almond kernel, thus reflecting the need of a careful choice of parents in a breeding program, where a noticeable increase of some chemical compounds may be attained. Moreover, the wide variability observed of the chemical components represents a very promising base to obtain new almond cultivars and superior genotypes with higher oil quality.

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## References

- ABDALLAH A., AHUMADA M.H., GRADZIEL T.M., 1998. Oil content and fatty acid composition of almond kernels from different genotypes and California production regions. J Amer Soc Hortic Sci 123, 1029-1033.
- ARTEAGA N., SOCIAS i COMPANY R., 2002. Heritability of fruit and kernel traits in almond. Acta Hortic 591, 269-274.
- BARBERA G., DI MARCO L., LA MANTIA T., SCHIRRA M., 1994. Effect of rootstock on productive and qualitative response of two almond varieties. Acta Hortic 373, 129-134.
- CHEN C.Y., LAPSLEY K., BLOOMBURG J., 2006. A nutrition and health perspective on almonds. J Sci Food Agric 86, 2245-2250.
- DAVIES C.S., NEILSON S.S., NEILSON N.C., 1987. Flavor improvement of soybean preparations by genetic renoval of lipoxygenase 2. J Amer Oil Chem Soc 6, 1428-1433.
- FALCONER D.S., 1970. Introduction to quantitative genetics. Roland Press Co, NY, USA.
- FELIPE A.J., 2000. El almendro: el material vegetal. Integrum, Lérida, Spain. [In Spanish].
- GOFFMAN F.D., BECKER H.C., 2001. Diallel analysis for tocopherol in seeds of rapeseed. Crop Sci 4, 1072-1079.
- GRASSELLY C., 1972. L'amandier: caractères morphologiques et physiologiques des variétés, modalité de leur transmission chez les hybrides de première génération. PhD thesis. Univ Bordeaux, France. [In French].
- HANSCHE P.E., BERES W., FORDE H.I., 1972. Estimate of quantitative genetic properties of walnut and their implication for cultivar improvement. J Amer Soc Hortic Sci 97, 279-285.
- ISO, 2000. ISO standard 5509. Animal and vegetable fats and oils – Preparation of methyl esters of fatty acids. In-

ternational Organization for Standardization, Geneva, Switzerland.

- KAMAL-ELDIN A., ANDERSON R., 1997. A multivariate study of the correlation between tocopherol and fatty acid in vegetable oils. J Amer Oil Chem Soc 74, 375-376.
- KESTER D.E., HANSCHE P.E., BERES W., ASAY R.N., 1977. Variance components and heritability of nut and kernel traits in almond. J Amer Soc Hortic Sci 102, 264-266.
- KODAD O., 2006. Criterios de selección y de evaluación de nuevas obtenciones autocompatibles en un programa de mejora genética del almendro (*Prunus amygadalus* Batsch). PhD thesis. Univ Lérida, Spain. [In Spanish].
- KODAD O., SOCIAS i COMPANY R., 2006. Phenotypic correlation between some agrochemical traits of the almond kernel. Acta Hortic 726, 259-264.
- KODAD O., SOCIAS i COMPANY R., 2008a. Variability of oil content and of major fatty acid composition in almond (*Prunus amygdalus* Batsch) and its relationship with kernel quality. J Agric Food Chem 56, 4096-4101.
- KODAD O., SOCIAS i COMPANY R., 2008b. Fruit quality in almond as related to the type of pollination in self-compatible genotypes. J Amer Soc Hortic Sci 133, 320-326.
- KODAD O., SOCIAS i COMPANY R., PRATS M.S., LÓPEZ ORTIZ M.C., 2006. Variability in tocopherol concentrations in almond oil and its use as a selection criterion in almond breeding. J Hortic Sci Biotechnol 81, 501-507.
- KODAD O., ESTOPAÑÁN G., JUAN T., SOCIAS i COMPANY R., 2009. Xenia effects on oil content and fatty acid and tocopherol concentrations in autogamous almond cultivars. J Agric Food Chem 57, 10809-10813.
- KODAD O., ESTOPAÑÁN G., JUAN T., MOLINO F., MAMOUNI A., MESSAOUDI Z., LAHLOU M., SOCIAS i COMPANY R., 2010. Plasticity and stability in the major fatty acid content of almond kernels grown under two Mediterranean climates. J Hortic Sci Biotechnol 85, 381-386.
- LEÓN L., UCEDA M., JIMÉNEZ A., MARTÍN L.M., RALLO L., 2004. Variability of fatty acid composition in olive (*Olea europaea* L.) progenies. Span J Agric Res 2(3), 353-359.
- LÓPEZ-ORTIZ M.C., PRATS-MOYA S., SANAHUJA A.B., MAESTRE-PÉREZ S.E., GRANÉ-TERUEL N., MARTÍN-CARRATALÁ M.L., 2008. Comparative study of tocopherol homologue content in four almond oil cultivars during two consecutive years. J Food Comp Anal 21, 144-151.
- MARAME F., DESSALEGNE L., FININSA C., SIGVALD R., 2009. Heterosis and heritability in crosses among Asian and Ethiopian parents of hot pepper genotypes. Euphytica 168, 235-247.
- MOTILVA M.J., RAMO T., ROMERO M.P., 2001. Caracterización geográfica de los aceites de oliva vírgenes de la denominación de origen protegida 'Les Garrigues' por su perfil de ácidos grasos. Grasas y Aceites 52, 26-32. [In Spanish].
- ROMOJARO F., RIQUELME B.F., GIMÉNEZ J.J., LLORENTE S., 1988. Fat content and oil characteristics of some almond varieties. Fruit Sci Rep 15, 53-57.

- SAURA CALIXTO F., CAÑELLAS MUT J., SOLER L., 1988. La almendra: composición, variedades, desarrollo y maduración. INIA, Madrid, Spain. [In Spanish].
- SOCIAS i COMPANY R., 1998. Fruit tree genetics at a turning point: the almond example. Theor Appl Genet 96, 588-601.
- SOCIAS i COMPANY R., KODAD O., ALONSO J.M., GRADZIEL T.M., 2008. Almond quality: a breeding perspective. Hortic Rev 34, 197-238.
- SOUZA V., BYRNE D.H., TAYLOR J.F., 1998. Heritability, genetic and phenotypic correlations, and predict

selection response of quantitative traits in peach. II. An analysis of several fruit traits. J Amer Soc Hortic Sci 123, 604-611.

- YAO Q., MEHLENBACHER A., 2000. Heritability, variance components and correlation of morphological and phenological traits in hazelnut. Plant Breed 119, 369-381.
- ZACHEO G., CAPELLO M.S., GALLO A., SANTINO A., CAPELLO A.R., 2000. Changes associated with postharvest ageing in almond seeds. Lebens-Wissens Technol 33, 415-423.