A NEW LATE BLOOMING ALMOND CULTIVAR

INTRODUCTION

The almond (*Prunus amygdalus* Batsch) breeding program of the CITA of Aragón aims to develop new self-compatible and late-blooming cultivars to solve the main problem detected in Spanish almond growing, its low productivity, due to the occurrence of frosts at blooming time or later and to a deficient pollination (Felipe, 2000). Now 'Vialfas' is being released because of its good horticultural and commercial traits, as well as its late-blooming time.

ORIGIN

'Vialfas' (selection I-3-27, clone 546) comes from the cross of 'Felisia', a self-compatible and late-blooming release of the Zaragoza breeding programme of small kernel size (Socias i Company and Felipe, 1999), and 'Bertina', a late-blooming local selection of large kernel size (Felipe, 2000). Consequently, 'Vialfas' is a full sib of 'Mardía'. This cross was made with the aim of utilizing two late blooming almond cultivars, one of them carrying the late-bloom allele *Lb* (Socias i Company *et al.*, 1999), of very different kernel size and genetically very distant, in order to avoid the problems related to inbreeding depression (Alonso and Socias i Company, 2007).

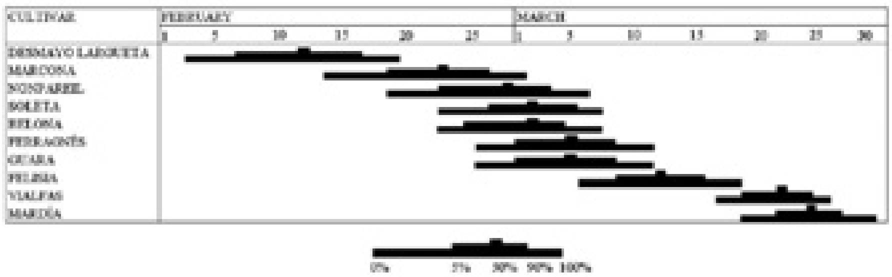
BLOOMING TIME

Blooming time has always been a very important evaluation trait in the CITA breeding program. As an average, 'Vialfas' blooming time is 22 days later than 'Nonpareil', 17 days after 'Guara', 10 days after 'Felisia', and three days before 'Mardía' (Fig. 1). The consistent late blooming time is due to very high chilling and heat requirements (Alonso *et al.*, 2005; Alonso and Socias i Company, 2009). Chilling requirements are similar to those of 'Mardía', but with slightly lower heat requirements (Table 1), which could explain the difference in blooming time. Flowers are of mean size, white, with peristigmatic style, both on spurs and on one-year shoots. Bloom density is high and consistent (Kodad and Socias i Company, 2008b).

AUTOGAMY

Self-compatibility was tested as soon as the original seedling produced the first flowers by examining the arrival or not of pollen tubes at the ovary after self-pollination. Sets after self-pollination and autogamy were studied on three grafted trees during four years due to the large variability found between years in field trials for fruit set (Socias i Company *et al.*, 2005). Average set after artificial self-pollination was 16.2%, not significantly different from cross-pollination, 16.9%, showing a good self-compatible behavior. These sets (Kodad and Socias i Company, 2008a) are lower than those considered for a commercial crop in Californian cultivars (Kester and Griggs, 1959), but ensure a good crop level because of the high bloom density of this selection, resulting in a high productivity (Kodad and Socias i Company, 2006). Its *S*-allele genotype has been determined as $S_{fi}S_{11}$ (Kodad and Socias i Company, 2008a).

Fig. 1. Mean flowering time of 'Vialfas' as related to other cultivars (7-years average). Percentages refer to the amount of flowers opened.



PERFORMANCE

Field behavior has been evaluated with three grafted trees in an experimental plot and in six trees in three external trials. One on the most important points considered was the behavior in relation to spring frost injury. Especially important were the observations in 2003 and 2004, with severe frosts in most almond growing regions of Spain. Whereas cultivars considered as resistant to frosts such as 'Guara' (Felipe, 1988) suffered important yield reductions, 'Vialfas', due to its very late blooming season, did not suffer any damage (Kodad and Socias i Company, 2005).

Tree training has been easy because of its slightly upright growth habit (Kodad and Socias i Company, 2008b), without the problem of bending branches of 'Guara' (Fig. 2). Adult trees show an intermediate vigor and branching intensity, as well as a good equilibrium between vegetative growth and production, thus allowing reduction of pruning. Field observations in the different locations for at least four years showed its tolerance to *Polystigma* and other fungal diseases, such as *Taphrina* and *Coryneum*.

Ripening time is early, about nine days later than 'Guara', the earliest ripening cultivar, thus allowing the succession of harvest. Nut fall before harvest has been very low, but nuts fell easily when shaken. Yield rating in a trial where different late-blooming cultivars and breeding selections were evaluated has been slightly lower than for 'Guara', 7.5 vs. 9 in a 0-9 scale (Alonso *et al.*, 2015), considering that 'Guara' is a very high-yielding cultivar and rated as 9 in this scale (Alonso *et al.*, 2012).

Table 1. Chilling and heat requirements of 'Vialfas' as related to other cultivars.

Cultivar	Chilling requirements (CU)[z]	Heat requirements (GDH)[y]
Desmayo Largueta	428	5,458
Marcona	428	6,603
Nonpareil	403	7,758
Belona	353	7,741
Soleta	340	7,872
Ferragnès	444	8,051
Guara	340	8,159
Felisia	329	9,465
Vialfas	503	10,066
Mardía	503	10,663

[z]Chilling units

[y]Growing Degree Hours in ºCelsius

Table 2. Protein and fat composition of 'Vialfas' kernels as compared to other cultivars.

Cultivar	Protein (% DW[z])	Oil (% DW[z])	Oleic acid (% oil)	Linoleic acid (% oil)	Oleic/linoleic acid ratio	Palmitic acid (% oil)	Stearic acid (% oil)	Palmitoleic acid (% oil)
D. Largueta	24.5	57.35	70.65	20.55	3.44	7.08	2.09	0.51
Marcona	23.8	59.10	71.75	19.40	3.70	6.15	2.09	0.52
Nonpareil	15.0	53.47	67.72	23.28	2.91	6.34	1.44	0.52
Belona	16.4	65.40	75.60	12.73	5.94	5.29	2.40	0.42
Soleta	20.0	61.80	69.20	19.70	3.51	6.40	1.65	0.60
Ferragnès	25.4	57.53	70.20	20.10	3.49	5.57	2.05	0.41
Guara	29.3	54.33	63.10	25.70	2.46	6.01	3.17	0.38
Felisia	27.0	56.32	68.05	22.10	3.08	5.90	1.75	0.60
Vialfas	18.8	57.37	77.97	12.32	6.33	5.70	2.48	0.58
Mardía	19.8	59.10	74.95	16.55	4.53	5.60	2.10	0.50

[z]Dry weight

Table 3. Tocopherol composition of 'Vialfas' kernels as compared to other cultivars.

Cultivar	α-tocopherol ($mg{\cdot}kg^{-1}$ oil)	γ-tocopherol ($mg{\cdot}kg^{-1}$ oil)	δ-tocopherol ($mg{\cdot}kg^{-1}$ oil)	Total tocopherol ($mg{\cdot}kg^{-1}$ oil)
D. Largueta	304.3	15.3	1.66	321.3
Marcona	463.3	18.5	1.87	483.7
Nonpareil	400.0	27.8	1.57	429.4
Belona	418.4	15.4	2.18	436.0
Soleta	214.0	13.3	1.51	228.8
Ferragnès	377.5	18.7	1.84	398.0
Guara	385.4	15.7	1.76	402.9
Felisia	250.6	18.2	1.73	270.6
Vialfas	222.5	14.0	1.53	238.0
Mardía	201.5	12.1	1.23	214.8

Fig. 2. 'Vialfas' tree in full production.

Table 4. Phytosterol compositionof 'Vialfas' kernels as compared to other cultivars.

Cultivar	Kernel phytosterol content ($mg{\cdot}kg^{-1}$ kernel)	Oil phytosterol content ($mg{\cdot}kg^{-1}$ oil)	β-Sitosterol (% phytosterols)	Δ^5-Avenasterol (% phytosterols)	Campesterol (% phytosterols)	Other phytosterols (% phytosterols)
D. Largueta	1445	2559	75.64	15.43	3.32	5.62
Marcona	2260	3515	74.69	13.95	2.11	9.24
Nonpareil	1891	3060	75.35	13.33	3.52	7.80
Belona	1848	3076	74.37	16.93	1.79	6.91
Soleta	1991	3321	68.44	22.40	1.80	7.37
Ferragnès	1911	2963	73.94	15.03	2.47	8.57
Guara	1506	2772	71.89	19.24	2.95	5.92
Felisia	1613	2792	69.72	17.74	3.64	8.91
Vialfas	1458	2589	68.65	19.35	3.36	8.63
Mardía	1531	2863	72.01	19.24	3.52	9.40

The external trials have shown its good adaptation to different growing and weather conditions, maintaining a high level of bud density in all locations (Kodad and Socias i Company, 2008b). A trial in Aniñón (Zaragoza) at 730 m above sea level and of very cold climate has had good production even in years with late frosts. A trial in Caspe (Zaragoza), at 100 m above sea level and with a milder climate, has shown their very good production as well as vegetation. Although 'Vialfas' showed similar unshelled nut production than Guara', the kernel production was slightly lower due to the lower shelling percentage of 'Vialfas' (Alonso *et al.*, 2015). Blooming and ripening dates observed in these locations have been, as expected, earlier in Caspe than in Zaragoza, but later in Aniñón.

Tree vigor, shown as trunk cross sectional area (TCSA) is low. Consequently, it could be better adapted to high density plantings than more vigorous cultivars. As a result of this low vigor, 'Vialfas' is in the group of cultivars with the highest productivities, 86 g of kernel cm^{-2} TCSA, similar to the most productive cultivar, 'Guara' (Alonso *et al.*, 2015).

Nut and fruit evaluation has been done trough seven years according to the IPGRI and UPOV descriptors. Nuts show a very good aspect and good size (4.7±0.5g), with a high number of small points, between elliptic and heart-shaped (Fig. 3).

Table 5. Mineral composition of 'Vialfas' kernels as compared to other cultivars.

Cultivar	Fiber (% DW^z)	Ash (% DW^z)	K (‰ DW^z)	Ca (‰ DW^z)	Mg (‰ DW^z)
D. Largueta	4.74	3.39	0.84	0.23	0.29
Marcona	4.39	3.09	0.75	0.19	0.28
Belona	4.43	2.67	0.82	0.10	0.26
Soleta	4.78	2.76	0.78	0.18	0.22
Ferragnès	5.60	3.46	0.86	0.27	0.27
Guara	4.58	3.28	0.85	0.25	0.28
Felisia	5.25	3.10	0.85	0.21	0.24
Vialfas	5.56	3.38	0.87	0.28	0.25
Mardía	5.54	3.17	0.86	0.29	0.24

^{z}Dry weight

Fig. 3. Nut and kernel of 'Vialfas'.

The shell is hard, adapted to the Spanish industry, with low shelling percentage (25%). However, the kernel percentage over the total fruit dry weight (DW) is 22.1%, quite high when compared with other cultivars: 23.1% for 'Guara', with the highest kernel percentage, and slightly less than 10% for 'Marcona', 'Desmayo Largueta' and 'Nonpareil' (Alonso *et al.*, 2012). Kernels also show a very good aspect and good size (1.2±0.2g), heart-shaped, without double kernels (Fig. 3). Industrial cracking has been carried out by the Cooperative "Frutos Secos Alcañiz" and has shown very good results, despite the presence of double layers in the shell. The chemical composition of the kernels has been determined in order to establish their best utilization opportunities. The content in protein is low and that of oil is medium, similar to that of 'Marcona' (Table 2), a very interesting trait for "turrón" (nougat) production. The percentage of oleic acid, that of higher quality for fat stability and nutritive value in the lipid fraction, is especially high (Kodad and Socias i Company, 2008c), close to 78% (Table 2). The content in linoleic acid, of lower quality than the oleic acid regarding oil stability, is low, showing the highest ratio of oleic/linoleic acids than any other cultivar (6.3), as another index of high oil quality. The amount of tocopherols (Table 3) is lower than in other cultivars (Kodad *et al.*, 2006), indicating the need for a rapid processing of kernels after cracking. The level of phytosterols (Table 4) is also low as compared to other cultivars (Fernández-Cuesta *et al.*, 2012).

The amount of fiber in kernel is particularly high, as well that of ash, being in both cases close to the highest amount of all cultivars analyzed (Table 5). Likewise the presence of mineral elements is very high for K and Ca, and average for Mg (Table 5).

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THE ORIGIN OF THE ALMOND 'GUARA'

ABSTRACT

'Guara' has been the most widely planted almond cultivar in Spain recently. The introducers of this cultivar reported its origin as unknown and suggested that was related to the Italian cultivar 'Tuono'. Indeed, farmers and researchers experience revealed close pomological similarities between 'Guara' and 'Tuono'. In order to compare the identity of the two cultivars, their genetic profiles (fingerprints) were determined with a set of 12 molecular markers (type microsatellites, SSR) used to analyze the INRA clones of 'Guara', 'Tuono', 'Supernova' and 'Mazzetto', the two last considered synonymous of 'Tuono'. In addition, a set of 23 SSRs was also analyzed in 'Guara' and 'Tuono' samples from different almond reference collections of CEBAS-CSIC (Murcia, Spain), INRA (Avignon, France), IRTA (Constantí, Spain), and the University of Bari (Bari, Italy). The results confirmed that 'Guara' and 'Tuono' present identical DNA fingerprints for the 35 SSRs analyzed. This information together with its unknown origin and the similar agronomic traits of the two cultivars, demonstrates that 'Guara' is identical to 'Tuono'.

Keywords: Almond, *Prunus dulcis*, Self-compatibility, Cultivar identification, Molecular markers, Microsatellites, SSRs

INTRODUCTION

The almond (*Prunus dulcis* Mill.) 'Guara' has been the most widely planted cultivar in Spain until recently (1996-2010), representing almost 40% of all new plantations (Socias i Company et al. 2009; 2011; 2012; Socias i Company and Couceiro, 2014). Its extensive use can be attributed to its fair agronomic performance, given that it was the first self-compatible late flowering cultivar available. In addition, crop development plans helped planting this variety, despite its intermediate vigour and productivity, slightly bitter kernel flavour, susceptibility to various fungus diseases and the significant percentage of double kernels. However, due to its production consistency and resistance to frost it is still planted in Spain (Socias i Company et al. 2011; 2012; Socias i Company and Couceiro, 2014).

'Guara' was named in 1987 at the "Unidad de Fruticultura" of SIA (now CITA) in Zaragoza (Spain) (Felipe and Socias i Company, 1987; Kester, 1994). According to its introducers, 'Guara' is of unknown origin and it came from a clonal and sanitary selection of an accession, mislabelled as 'Cristomorto', introduced in the SIA almond collection in 1970 (Felipe, 2017). In CITA's breeding programme, besides 'Guara', eight other almonds have been released ('Aylés', 'Moncayo', 'Blanquerna', 'Cambra', 'Felisia', 'Soleta', 'Belona' and 'Mardía'). However, according to information provided by the breeders, of the nine releases, 'Guara' represented some 95% of CITA cultivar plants propagated in nurseries between 1996 and 2010 (Socias i Company et al. 2011, 2012, Socias i Company and Couceiro, 2014). Recently, also 'Vialfas' was released.

'Guara' was registered in the "Spanish Register of Protected Varieties" in 2003, and later cancelled in 2012 (Boletín Oficial del Estado Español, 2003 and 2012), despite the wide dissemination of the cultivar in Spain during the nine years under registration (Socias i Company et al., 2012, Socias i Company and Couceiro, 2014).

For a long time, researchers, technicians and farmers have observed similar traits and agronomic behaviour in 'Guara' and the Italian cultivar 'Tuono' (flowering time, self-compatibility, productivity, ripening time, tree vigour, fruit shape, fruit flavour and fungus disease susceptibility) (Grasselly and Duval 1987, Navarro 2002, Muncharaz 2004, Arquero et al., 2008, Arquero 2013). For this reason, together with the unknown origin of the cultivar, different researchers have suspected that 'Guara' might be the native Italian 'Tuono'.

'Tuono' is one of the most important local varieties in Italy. It first spread in Apulia around 1830, in the territory of Trinitapoli (Foggia province), where it was the only cultivar grown (Pantanelli and Fanelli, 1934). In 1970, this cultivar represented 10–40% of the plantations in these areas, depending on location (Grasselly and Crossa-Raynaud, 1980). It was later introduced to Greece where it is known as 'Truoito' (Stylianides, 1976) and to Libya and Tunisia where it is known as 'Mazzetto' (Grasselly and Olivier, 1976). Currently, most of the new almond orchards in southern Italy are planted with 'Tuono' due to its late blooming, self-fertility and wide environmental adaptability (Sottile et al., 2014). Interestingly, the cultivar 'Supernova', released as a self-compatible mutant of 'Fascionello', was found to be 'Tuono' (Marchese et al., 2008).