Fruit Quality in Almond as Related to the Type of Pollination in Self-compatible Genotypes

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Abstract. Nut and kernel dimensions and sphericity, shelling percentage, oil content, and fatty acid composition were studied over 2 years in 15 advanced almond (Prunus amygdalus Batsch) selections. The aim was to test the effect of pollination type on these fruit traits for this group of new self-compatible selections of a mostly self-incompatible species as well as the yearly effect on these variables. Variability between selections was much higher than that between years, showing a moderate level of year-stability and a significant year effect only for some variables. The different pollination treatments affected all chemical components studied, as well as nut and kernel weight, but not the other physical traits. Self-pollination decreased kernel weight and volume as well as oil content and percentage of linoleic acid but increased the percentage of oleic acid. These variations in the fatty acid composition were in the trend of increasing kernel quality. Inbreeding depression could also negatively affect several aspects of nut and kernel quality. Thus, autogamous almond genotypes without apparent symptoms of inbreeding depression may yield kernels of increased nutritional and industrial quality.

Most almond breeding programs have fostered the development of self-compatible cultivars to overcome the problems related to cross-pollination of this mostly self-incompatible species (Socias i Company, 2002). Consequently, self-compatibility has been the primary trait considered during evaluation of seedlings in a breeding program (Socias i Company et al., 1998). However, any new self-compatible cultivar must meet all horticultural and commercial requirements to be valuable for the grower and the industry. The main efforts in evaluating new self-compatible almond genotypes have been directed toward establishing their actual self-compatibility level. The first attempts focused on pollen tube growth, to ensure that this growth after self-pollination was similar to that after cross-pollination with a cross-compatible pollen and, thus, to confer a good self-compatibility level (Socias i Company et al., 1976). Additionally, this good pollen tube growth after self-pollination had to result in similar fruit sets, which may not always be the case (Socias i Company and Felipe, 1987). Furthermore, fruit set should reach the level of a commercial crop (Socias i Company and Felipe, 1992). From a commercial point of view, self-compatibility must consider not only the crop level but also that this crop must be of an acceptable quality. However, this aspect has not yet received much attention, probably because standards of almond fruit quality have been based on physical parameters (Socias i Company et al., 1998) and because differences in consumer preferences make a universal definition of fruit quality difficult, even ephemeral (Janick, 2005; Socias i Company et al., 2008).

Very few studies have considered almond fruit characteristics in relation to pollination type. Fruit development can be irregular after self-pollination (Grasselly and Olivier, 1988), resulting in smaller kernels (Torre Grossa et al., 1994). Thus, all these negative effects must be considered and evaluated before any new cultivar release. Although Torre Grossa et al. (1994) remarked the negative effect of self-pollination of ‘Lauranne’ in its final fruit size in a single year, Legave et al. (1997) did not find any difference in this same cultivar during 3 years. Dicenta et al. (2002) included this and five other genotypes in their 1-year study, and found no difference between self- and cross-pollination for several fruit traits, including fruit weight, kernel weight, shelling percentage, double kernels, empty nuts, and split kernels. All these studies have considered only physical traits, but there is not yet any information on the chemical composition of the kernels as a function of pollination type. However, kernel composition in relation to its possible industrial utilization (Alessandroni, 1980) or quality stability (Kester et al., 1980) must be taken into account when evaluating the actual effect of self-compatibility on the agronomical and commercial qualities of any new cultivar.

As a consequence, our objective was to ascertain the effect of each type of pollination not only on the physical traits of the fruit and the kernel but also on the kernel chemical traits in several almond genotypes previously selected because of their high self-compatibility level. This information will be essential in evaluating the fruit and kernel quality of any new self-compatible cultivar—before further planting is recommended—to ensure a high-quality crop under all growing and pollination conditions.

Materials and Methods

Sixteen advanced selections coming from the cross between ‘Bertina’, a Spanish local selection, and ‘Felisia’, a release from the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) breeding program, were included in this study.
These selections were grafted onto the peach \textit{Prunus persica (L.) Batsch} × almond hybrid rootstock ‘Garnem’ and grown in blocks of three trees in an alluvial loamy soil.

Nuts were harvested at maturity, when fruit mesocarp was fully dried and split along the fruit suture and peduncle abscission was complete (Felipe, 1977). During two consecutive years, a sample of 20 fruit was collected randomly around the canopy from a plant after open pollination to study the stability of the studied traits for each genotype over the 2 years. Additionally, samples of 20 fruit were collected after three different pollination treatments—open pollination, manual self-pollination, and cross-pollination with ‘Cavaliera’ pollen—to ascertain the effect of each type of pollen on expression of the studied traits. As much as possible, homogeneous branches were used for artificial pollinations, and the different treatments were applied to two branches as described by Socias i Company et al. (2004).

Length, width, and thickness were measured with a precision of 0.01 mm in all nuts with a digital caliper. After measurements, nuts were cracked to obtain the kernel and determine the shelling percentage by weight using an electronic balance. Length, width, and thickness were similarly measured in all kernels. These variables allowed determination of the sphericity index (geometric diameter/length) of fruit and kernel, which is used to define their shape.

For chemical analysis, two replicates of 20 fruit of each genotype were randomly collected and dried for 2 d. After they had been blanched, the kernels were ground in an electric grinder. Oil was extracted from 4–5 g of ground almond kernels in a commercial fat extractor (Selecta, Barcelona, Spain) for 2 h using petroleum ether as solvent and keeping the heating source at 135 °C. Fat content was expressed as the difference in weight year to year was significant for oil content (Table 4). As expected, oil content and fatty acid composition varied significantly among genotypes, agreeing with the results of Abdallah et al. (1998) in California almond cultivars.

Oil content from 2005 harvest was slightly higher than that from 2004 (Table 2), with significant differences among years (Table 4). The genotype effect was significant for the different fatty acids, showing the particular fatty acid profile of each selection. The major differences in fatty acid composition among genotypes were observed for oleic (C18:1) and linoleic (C18:2) acids. The year effect differed depending on the fatty acid, being highly significant for oleic and palmitic acids, less significant for linoleic acid, and not significant for stearic acid. However, some selections did not show significant fluctuations between the 2 years (Table 3).

**Pollen effect on nut and kernel traits.** Nut and kernel weight, but not other physical traits, showed significant differences between different pollination types (Table 3), although mean LSD separation indicated that the values of the different traits were slightly higher after cross-pollination than after self-pollination (Table 2). Some selections showed differences between treatments higher than 20% (data not shown), mainly for the more variable traits of nut and kernel weight. The differences found between the two artificial pollinations and open pollination were also significant only for nut and kernel weight. The interaction genotype × treatment was significant for all studied traits, except for shelling percentage and kernel sphericity (Table 3), showing that the values of the physical traits of the fruit coming from the different pollination types change their range depending on each selection.

The interaction year × treatment was significant for fruit and kernel weight and shelling percentage but not for sphericity index (Table 3), thus indicating the important role of the year conditions in determining the physical traits of the nut independently of pollination type. The year effect was not significant for nut shape. On the other hand, despite the significance of the interaction year × treatment, values of in-shell and kernel weights after self-pollination were lower than those obtained after cross-pollination. In 2005, the comparison of the percentage of defective kernels between treatments showed that the highest values were obtained after self-pollination and the lowest ones after cross-pollination (Table 2).

Analysis of variance showed a significant effect of type of pollination on kernel chemical composition (Table 4), indicating a possible influence of pollen origin on almond kernel quality. Kernel taste did not show any difference between treatments (data not shown), but oil content and percentages of the different fatty acids were significantly affected by pollination type (Table 2). In general, cross-pollination slightly increased the percentage of kernel oil content, as it happened with the physical traits. However, in some selections, the oil percentage was higher in kernels coming from self-pollination. In most selections, self-pollinated kernels showed higher oleic acid content and lower linoleic acid content than did cross-pollinated kernels. The interaction genotype × treatment was significant for all the studied traits (Table 4), showing that their range of values may change depending on each selection.
effect of the pollen source on oleic acid content was verified at the individual level, showing that the self-pollinated kernels had higher amounts of oleic acid (Table 2). This situation was maintained in both years. Conversely, the significance of the interaction genotype × treatment × year showed that variations in oil content and in oleic acid percentage of each genotype were affected by external conditions independently of the treatment.

**Discussion**

**VARIABILITY.** The significance of the genotype effect on the physical traits considered agreed with the results previously reported (Dicenta et al., 1993; Kester et al., 1977). However, the significant year effect on fruit and kernel size is probably due to the non- or less-significant year effect on the nut and kernel sphericity index, used to evaluate their shape, showed that shape is constant over the years, thus being reasonably considered a cultivar trait (Gülcen, 1985). However, the differences for kernel weight between years may not be important from a commercial point of view because most genotypes present kernel weights higher than 1 g, considered an objective in almond breeding programs.

In addition to the year-to-year fluctuation of these physical traits, there is a significant year × genotype interaction (Table 3), indicating that changes in the environmental conditions because a strong environmental and seasonal component has been described for fruit and kernel (Dicenta et al., 1993), as well as for crop load, tree vigor, and soil moisture (Kester and Gradziel, 1996). The non- or less-significant year effect on the nut and kernel sphericity index, used to evaluate their shape, showed that shape is constant over the years, thus being reasonably considered a cultivar trait (Gülcen, 1985). However, the differences for kernel weight between years may not be important from a commercial point of view because most genotypes present kernel weights higher than 1 g, considered an objective in almond breeding programs.

**Table 2. Average values of physical and chemical nut and kernel traits of 15 almond selections.**

| Selection | Physical traits | Chemical traits |
|-----------|----------------|----------------|
| In-shell wt (g) | Kernel wt (g) | Shelling percentage (%) | Fruit sphericity | Kernel sphericity | Defective kernels (%) | Oil content (% dry wt) | Oleic acid (% oil) | Linoleic acid (% oil) | Palmitic acid (% oil) | Stearic acid (% oil) |
| G-1-1 | 4.33 | 1.09 | 27.03 | 0.65 | 0.51 | 18.00 | 59.59 | 76.24 | 14.39 | 6.26 | 2.12 |
| G-1-23 | 3.45 | 0.85 | 25.05 | 0.68 | 0.56 | 13.00 | 61.23 | 37.10 | 14.57 | 5.47 | 1.89 |
| G-1-4 | 4.10 | 1.04 | 25.76 | 0.70 | 0.56 | 19.40 | 59.72 | 76.20 | 13.77 | 6.27 | 1.59 |
| G-2-1 | 5.43 | 1.23 | 23.22 | 0.67 | 0.53 | 13.80 | 55.17 | 74.10 | 16.81 | 5.96 | 1.85 |
| G-2-2 | 3.64 | 1.01 | 28.32 | 0.65 | 0.54 | 8.33 | 55.46 | 75.77 | 15.04 | 6.32 | 1.52 |
| G-2-22 | 3.21 | 0.91 | 29.99 | 0.70 | 0.59 | 5.40 | 59.46 | 76.44 | 26.35 | 6.39 | 1.59 |
| G-3-3 | 4.11 | 1.17 | 26.17 | 0.69 | 0.56 | 6.80 | 55.73 | 74.04 | 15.93 | 6.31 | 1.90 |
| G-3-4 | 5.96 | 1.23 | 23.22 | 0.67 | 0.53 | 12.00 | 56.52 | 75.86 | 15.28 | 5.86 | 1.48 |
| G-3-5 | 4.56 | 1.23 | 24.30 | 0.67 | 0.55 | 5.68 | 58.36 | 77.46 | 14.28 | 6.01 | 1.47 |
| G-3-8 | 3.98 | 1.04 | 25.22 | 0.72 | 0.55 | 9.12 | 57.55 | 76.45 | 15.65 | 6.31 | 1.90 |
| G-3-14 | 3.96 | 1.09 | 25.83 | 0.72 | 0.57 | 6.40 | 58.62 | 76.70 | 14.22 | 6.10 | 1.43 |
| I-3-27 | 4.18 | 1.06 | 26.69 | 0.71 | 0.58 | 7.69 | 60.01 | 75.54 | 13.96 | 6.65 | 2.27 |

Table 1. Two-year average values of physical and chemical nut and kernel traits of 15 almond selections depending on type of pollination and year of study.

| Trait | 2004 | 2005 |
|-------|------|------|
| Physical traits | Treatment | Treatment |
| In-shell wt (g) | 4.18 b | 4.18 a | 4.17 a | 4.18 b |
| Kernel wt (g) | 1.07 b | 1.07 a | 1.07 a | 1.07 b |
| Shelling percentage (%) | 25.89 a | 26.37 a | 26.15 a | 25.98 a |
| Fruit sphericity | 0.69 a | 0.70 a | 0.70 a | 0.69 a |
| Kernel sphericity | 0.55 a | 0.55 a | 0.55 a | 0.55 a |
| Defective kernels (%) | — | — | — | — |
| Chemical traits | Oil content (% dry wt) | 57.35 b | 57.10 a | 57.24 a | 57.12 b |
| | Palmitic acid (% oil) | 5.68 b | 5.68 a | 5.68 a | 5.68 b |
| | Palmitoleic acid (% oil) | 0.48 b | 0.48 a | 0.48 a | 0.48 b |
| | Stearic acid (% oil) | 1.66 c | 1.66 b | 1.66 b | 1.66 c |
| | Oleic acid (% oil) | 76.45 a | 76.45 a | 76.45 a | 76.45 a |
| | Linoleic acid (% oil) | 15.01 b | 15.01 a | 15.01 a | 15.01 b |

* = self-pollination, × = cross-pollination, open pollination.

* Mean separation for each trait by LSD multiple-range test at P ≤ 0.05.
Table 3. Analysis of variance of physical nut and kernel traits of 15 almond selections.

| Source of variation          | df  | In-shell wt | Kernel wt | Shelling percentage | Fruit sphericity | Kernel sphericity |
|-----------------------------|-----|-------------|-----------|---------------------|------------------|-------------------|
| Genotype                    | 14  | 86.41***    | 4.80***   | 3297.5***           | 0.115***         | 0.091***          |
| Year                        | 1   | 31.32**     | 0.08**    | 2178.5***           | 0.383***         | 0.398***          |
| Genotype × year             | 14  | 11.92***    | 1.12***   | 3360.2***           | 0.109***         | 0.012***          |
| Treatment                   | 2   | 09.75***    | 0.81***   | 3311.1***           | 0.009***         | 0.003***          |
| Genotype × treatment        | 28  | 42.64***    | 0.19***   | 3321.9***           | 0.002***         | 0.002***          |
| Year × treatment            | 2   | 46.74***    | 0.73***   | 3366.3*             | 0.001***         | 0.004***          |
| Genotype × year × treatment | 28  | 434.9***    | 0.23***   | 3370.9***           | 0.002***         | 0.001***          |
| Residual                    | 1710| 0.31        | 0.025     | 21.76               | 0.0006           | 0.002             |

Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Table 4. Analysis of variance of fatty composition of 15 almond selections.

| Source of variation          | df  | Oil content | Palmitic acid | Stearic acid | Oleic acid | Linoleic acid |
|-----------------------------|-----|-------------|---------------|-------------|-----------|--------------|
| Genotype                    | 14  | 40.3***     | 0.97***       | 0.91***     | 105.5***  | 89.9***      |
| Year                        | 1   | 27.7***     | 19.3**        | 0.01***     | 74.44***  | 28.8***      |
| Genotype × year             | 14  | 58.9***     | 11.1***       | 0.29***     | 059.5***  | 51.4***      |
| Treatment                   | 2   | 58.9***     | 11.1***       | 0.29***     | 059.5***  | 51.4***      |
| Genotype × year × treatment | 28  | 3.4***      | 3.91***       | 0.21***     | 004.24*** | 16.5***      |
| Year × treatment            | 2   | 3.4***      | 3.91***       | 0.21***     | 004.24*** | 16.5***      |
| Genotype × year × treatment | 28  | 3.4***      | 3.91***       | 0.21***     | 004.24*** | 16.5***      |
| Residual                    | 179 | 1.72        | 0.12          | 0.015       | 0.61      | 0.71         |

Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

This correlation is due to an undefined environmental condition in 2004 and not to genetics. Another significant correlation was that of kernel weight and oil content in both years, but only for the open pollination treatment (Table 5). Abdallah et al. (1998) also reported a low correlation between kernel weight and oil content. However, only correlation coefficients higher than 0.71 or lower than −0.71 have been suggested to be biologically meaningful (Skinner et al., 1999), and in our results, all correlation coefficients were lower than 0.5, thus showing their low biological value.

These results show that kernel oil and fatty acid composition present a higher year-to-year stability than do physical traits, indicating that the chemical components depend more on genotype than on environmental conditions. From a breeding point of view, some selections (G-2-25, G-3-3, and I-3-27) showed a very good performance during the 2 years of study, thus offering opportunities for optimizing oil content and quality through genotypic selection.

**Pollen effects on physical traits.** The pollen effect was significant on nut and kernel weight, with fruit coming from cross-pollination being heavier than that from self-pollination. The same results were found in other almond cultivars (Oukabli et al., 2002; Torre Grossa et al., 1994) and in other species, such as pecan [Carya illinoinensis (Wangenh.) K. Koch (Marquard, 1988)] and lychee [Litchi chinensis Sonn. (Stern et al., 1993)]. However, Legave et al. (1997) reported no negative effect of the self-pollen on the nut and kernel weight in ‘Lauranne’, in contrast to the results of Torre Grossa et al. (1994) in the same cultivar. Dicka et al. (2002) reported no significant differences between self- and cross-pollination for nut and kernel weight in six self-compatibles genotypes, although they found that kernel weight after cross-pollination was heavier (1.25 g) than after self-pollination (1.18 g).

The same discrepancy was found when comparing the nut and kernel physical traits in fruit coming from the two pollination types. Vargas et al. (2005) reported that kernels from open pollination were heavier and larger than selfed nuts, agreeing with our results, although Ortega et al. (2006) found significant differences between open and self-pollination for nut weight but not for kernel weight. In addition, they found a significantly higher number of defective kernels after self-pollination than after open pollination, although they did conclude that self-pollination did not have any effect on fruit quality. The cited results were from a single year, but our results were not consistent over both years. In the first year, nut and kernel were heavier after cross-pollination, and in the second year, no differences were found for nut weight. This explains the significance of the interaction year × treatment. This situation could be due to the unknown ratio of selfed fruit in the open pollination treatment, because these selections show a high level of autogamy (Kodad, 2006). The pollen donor effect could explain these results; note that Vezvaei and Jackson (1995) reported that heavier nuts were produced by ‘Price’, which produces small nuts, when pollinated by ‘Keane’, a large-kernelled cultivar. In pistachio (Pistacia vera L.) and chestnut (Castanea sativa Mill.), pollen from a small-seeded cultivar or species produced a decreased nut size when applied to a cultivar or species normally producing large seeds (Crane and Iwakiri, 1980). Rahemi and Mojadad (2001) reported that a pollen mixture of different cultivars affects significantly nut and kernel weight in hazelnut (Corylus avellana L.), stressing the importance of pollen on fruit characteristics.

The decrease in almond nut and kernel weight when comparing self- with cross-pollination has been attributed to inbreeding depression effects (Oukabli et al., 2002). The same hypothesis has been advanced in pear [Pyrus communis L. (Bell et al., 1981)]. Pollen source has been reported to affect the physical nut and kernel traits in self-incompatible cultivars (Eti et al., 1994; Kumar and Das, 1996; Vezvaei and Jackson, 1995),
where self-pollination cannot be applied, indicating the existence of xenia effects in almond (Kumar and Das, 1996). In macadamia (Macadamia integrifolia Maiden & Betche), mean fruit weight from mixed orchards of two or more cultivars is 14% higher than that from monocultivar orchards (Ito and Hamilton, 1980). These differences have been attributed to the positive effect of cross-pollination and not to inbreeding.

Cross-pollination has been considered to have a positive effect on the physical fruit characteristics in mandarin (Citrus reticulata Blanco) when compared with self-pollination (Vithanage, 1991; Wallace and Lee, 1999), and attributed to xenia (Wallace and Lee, 1999) because its own pollen did not affect the fruit weight from mixed orchards of two or more cultivars. In almond, however, this effect was lower than that from monocultivar orchards (Ito and Hamilton, 1980). These differences have been attributed to the positive effect of cross-pollination and not to inbreeding.

The effects of pollen on nut and kernel characteristics are known to occur in several nut crops. In almond, however, this pollen has been also reported not to affect nut and kernel shape in hazelnut (Rahemi and Mojadad, 2001).

**Pollen effect on chemical traits.** There were no differences in kernel taste between treatments, confirming that pollen type does not affect taste (Dicenta et al., 2000). However, the studies on the biosynthesis of the different chemical components of the almond kernel have been only focused on the process of accumulation of the different chemical fractions during fruit development (Saura Calixto et al., 1988) without considering the origin of these components. Dure (1975) pointed out that, during embryogenesis, in addition to the nutrients supplied by the mother plant, the embryo itself also synthesizes and stores an important fraction of nutrients. Saura Calixto et al. (1988) reported that oil accumulation takes place only when the cotyledon reaches a certain maturity level. This fact may suggest that the embryo contributes to oil kernel composition, thus being affected by both maternal and paternal genotypes.

The effect of self-pollination on chemical traits of the almond kernel has not yet been studied. Consequently, our results cannot be compared. However, Vezvaei and Jackson (1995) did not find any significant effect of pollen source on the chemical composition of Nonpareil kernels. In other species, such as corn (Zea mays L.), some chemical components of the endosperm (Correns, 1901) and of the embryo (Curtis et al., 1956) are highly affected by the pollen origin. A higher protein content was found in corn kernels after self-pollination than when compared with cross-pollination (East and Jones, 1920).

Although oil content in almond kernels obtained after self-pollination was lower than that from cross-pollination, the significant interaction of genotype × treatment indicates that the genotype response to the different treatments depends on the specific characteristics of each genotype.

The fatty acid profile showed significant differences depending on the pollination treatment. Mean separation by LSD test showed an increase of all fatty acids, with the exception of oleic and stearic acids, after cross-pollination. These differences could be due to the accumulation of additive genes after self-pollination because these traits seem to be quantitative (Kester and Gradziel, 1996). In mandarin, fruit of Ellonor after self-pollination showed a lower sugar content than after cross-pollination (Wallace and Lee, 1999). The decrease in oleic acid percentage after cross-pollination agrees with the increase in linoleic acid because of the negative correlation between both percentages (Abdallah et al., 1998). In any case, the increase in oleic acid and the decrease in linoleic acid contents in self-pollinated kernels confer to them a higher nutritional value and a better stability against rancidity than kernels from open and cross-pollination. As a consequence, autogamous cultivars in solid orchards could supply a product of the highest nutritional and technological quality to the commercial and industrial sectors.

**Conclusion**

The effects of pollen on nut and kernel characteristics are known to occur in several nut crops. In almond, however, this
phenomenon is under discussion. Our results confirm the significant effect of the type of pollen on the physical and chemical characteristics of almond fruit and show for the first time the possible positive effect of self-pollination on the fatty acid composition of the almond kernel, mainly on the increase of oleic acid content. The sharp decrease in weight and volume of the kernel after self-pollination in some genotypes points to the need for considering the possible effect of inbreeding on the expression of these traits, requiring careful evaluation in breeding programs where a reduced genetic pool of parents has been used as the source of self-compatibility and other horticultural and commercial traits (Kester et al., 1990; Socias i Company, 2002). Consequently, selection of autogamous cultivars without apparent symptoms of inbreeding depression must not affect kernel quality in almond but instead improve its nutritional and industrial quality.

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