Genetic analysis for physical nut traits in almond

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Abstract Almond breeding is increasingly taking into account kernel quality as a breeding objective. Although information on nut and kernel physical parameters involved in almond quality has already been compiled, the genetic control of these traits has not been studied. This genetic information would improve the efficacy of almond breeding programs. A linkage map with 56 SSR markers was constructed for the 'Vivot' × 'Blanquerna' almond population showing a wide range of variability for the physical parameters of nut and kernel. A total of 14 putative QTLs controlling these physical traits were detected in the current study, corresponding to six genomic regions of the eight almond linkage groups (LG). Some QTLs co-located in the same region or shared the same molecular markers, in a manner that reflects the correlations between the physical traits, as well as with the chemical components of the almond kernel. The LOD values for any given trait ranged from 2.06 to 5.17, explaining between 13.0 to 44.0% of the phenotypic variance of the trait. This new genetic information needs to be taken into account when breeding for physical traits in almond. Increases in the positive quality traits, both physical and chemical, need to be considered simultaneously whenever they are genetically independent, even if they are negatively correlated. This is the first complete genetic framework map for physical components of almond nut and kernel, with 14 putative QTLs associated with a large number of parameters controlling physical traits in almond.

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Key words Breeding · *Prunus amygdalus* Batsch · Nut traits · Kernel traits · QTL analysis · Genomics

Introduction

Almond [*Prunus amygdalus* Batsch, syn. *P. dulcis* (Mill.) D.A. Webb] is a major tree nut grown in areas with Mediterranean climate. As in any other crop, fruit quality is an important breeding goal despite the difficulties in defining a quality ideotype due to differences in consumer preferences (Janick 2005). Although quality is often related to the chemical composition of any fruit, including the nutritional and health aspects involved in defining its final value, some physical parameters must also be taken into account when evaluating quality. The physical traits of almond nut do not affect the organoleptic characteristics of the kernel, but have a special importance in the industry because of the different steps involved in almond processing (Socias i Company et al. 2008).

Almond breeding has until recently focused on selecting self-compatible and late-blooming cultivars with excellent physical attributes (Socias i Company et al. 2012). In addition to the sweet/bitter taste, the physical parameters were the only ones so far considered in almond evaluation, and their heritabilities determined (Kester et al. 1977). However, the physical traits of nuts and kernels were only considered as morphological traits for almond cultivar characterization, but not as quality traits. Some physical traits, however, require a more detailed examination, especially those related to the shell, since the shell has never been considered as a component of almond quality (Socias i Company et al. 2009).

The shell was probably neglected because it is not related to the chemical composition or to the organoleptic quality of the kernel. Nevertheless, the shell plays an important role during harvest and industrial processing and therefore should be taken into account when evaluating an almond cultivar. Soft-shell cultivars possess such a soft

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and thin shell that sometimes is not well sealed through the suture line, where abortion of the secondary ovule has taken place (Gradziel and Martínez-Gómez 2002), leaving an entry point for dust, insects and fungi. This contamination may be further aggravated by the presence of *Aspergillus* among the contaminating fungi and the production of the toxic aflatoxins, and other carcinogenic and immunosuppressive mycotoxins (Dicenta et al. 2002; Gradziel and Wang 1994). However, depending on the industry of each region, a different type of shell is preferred, hard in most Mediterranean countries and soft in California.

The size and shape of the nut must be taken into account for designing and adjusting appropriate technologies for harvesting, dehulling, transporting, classifying, processing and storing the crop. Additionally the size and shape of the kernel may define its utilization in specific commodities, such as chocolate bars, sugared almonds and sliced kernels. Although the physical parameters of the nut and the kernel have been scarcely considered as an objective in almond breeding, their relevance stresses the need to consider them as part of the evaluation criteria for almond quality in a breeding program (Socias i Company et al. 2009).

Among the physical traits, in addition to the attractiveness of the kernel, only shell hardness and the presence of double kernels have received some attention, mainly because of the specific requirements of the cracking process. Although the heritability of most of the physical parameters of almond nut and kernels is already known (see Socias i Company et al. 2012 for a review), not much is known about the genetics of these traits. Only the phenotypic correlation among some traits has been studied (Sánchez-Pérez et al. 2007a) and the independence of the physical and chemical traits has been established (Kodad and Socias i Company 2006). This scarce information requires deeper genetic examination in order to fully utilize these traits in a breeding program.

SSR (simple-sequence repeat or microsatellite) markers have recently become a very useful tool for constructing linkage maps and for locating genes controlling phenotypic variability.The development of markers associated with a trait may improve the speed and precision of breeding programs with the aim of selecting for this trait by marker-assisted selection. The first attempt to map agronomic traits in almond was undertaken by Sánchez-Pérez et al. (2007b), but these authors studied only a few physical traits including nut and kernel weight and shell hardness. The availability of the almond cross 'Vivot' × 'Blanquerna' ('V × B') made it possible for us to generate a linkage map of this population (Fernández i Martí et al. 2011), as well as determine 20 physical parameters of nuts and kernels (Font i Forcada 2008). Our objective in the present study was to identify QTLs associated with physical parameters of almond nut and kernel in order to develop a genetic framework for use in an almond breeding program to improve the physical quality of almond.

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Materials and methods

Plant material and DNA isolation

The offspring studied include 62 individuals from the cross 'V × B' obtained in the CITA almond breeding program of Zaragoza, Spain. The female parent 'Vivot' is a Spanish local cultivar, and the male parent 'Blanquerna' is a release from this program, obtained from 'Genco' × 'AS-1' pollination (Socias i Company and Felipe 1999;

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Fernández i Martí et al. 2009). These parents were selected because of their interesting characteristics, such as nut quality and medium-late blooming (Felipe 2000). This progeny is maintained as living plants in a nursery row using standard management practices, close to the parents, which belong to the Spanish almond germplasm collection located at 41°38'N and 0°53'W, at 220 m above sea level, at Zaragoza, Spain. The total genomic DNA was isolated using the procedure described by Doyle and Doyle (1987). The DNA was quantified and diluted to 10 ng μL^{-1} for PCR amplifications.

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Physical determinations

The physical parameters selected for measurement were those significant for almond processing (Aydin 2003). Fifty mature fruits were collected at random 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 nuts were left at room temperature for 2-3 weeks, as described by Font i Forcada et al. (2011). After taking nut measurements, shells were cracked to obtain the kernel. Nut and kernel weights were obtained using an electronic balance. The lineal parameters, length (L), width (W), and thickness (T), were measured with a digital calliper with a precision of 0.01 mm. These variables allowed to determine the W/L, T/L and L/W ratios, the size (L x W x T), the geometric diameter $(L \times W \times T)^{1/3}$, and the spherical index (geometric diameter/L). These parameters were determined both for the nut and the kernel. The average values of the results of two years were used for analysis. The absence of any year effect was confirmed by the lack of significant differences between the values of the two years.

DNA marker genotyping, genetic mapping and QTL analysis

A total of 110 SSR markers previously described in other *Prunus* species (Table 1) were tested in the 'V × B' almond progeny to identify polymorphic markers between the two parents, providing a good coverage of the *Prunus* bin mapping 'T × E' (Howad et al. 2005). Those heterozygous in one or both parents and resulting in a good coverage of the 'T × E' *Prunus* reference map were selected for analysis in the whole population. From the initial 'V × B' map (Fernández i Martí et al. 2011), eight SSRs designed from other *Prunus* species were additionally PCR amplified in order to be included in the previous map, using the same conditions (Table 1). Among these eight SSR, only four, the heterozygous ones, were placed in the map (CPPCT022, CPDCT027, BPPCT015 and CPPCT058).

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PCR reactions were performed in a 10 μL volume and the reaction mixture contained 1× PCR buffer (Invitrogen, Barcelona, Spain), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM of each primer, one unit of Taq DNA Polymerase (Invitrogen) and 20 ng of genomic DNA. The cycling parameters include denaturation for 1 min at 94ºC, 35 cycles of 15 s at 94ºC, 15 s for the corresponding annealing temperatures and 1 min at 72ºC, followed by a final extension of 2 min at 72ºC. The PCR reactions were carried out in a 96-well block Thermal cycler (Applied Biosystems, Madrid, Spain). PCR products were detected using an ABI PRISM 3130xl Genetic Analyzer and GeneMapper analysis software (Applied Biosystems). For capillary electrophoresis detection, forward SSR primers were labelled with 5'-fluorescence dyes PET, NED, VIC and 6-FAM and the size standard used in the sequencer was Gene Scan™ 500 Liz® (Applied Biosystems).

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We constructed a map for each parent, as if they were backcross one segregations using directly the markers segregating 1:1, converting the 1:1:1:1 into two 1:1 segregations (one for each parent) and using only the two homozygous classes of the 1:2:1 segregations. Composite interval mapping was used for mapping QTLs (MapQTL 4.0) (Van Ooijen et al. 2002). The LOD threshold of ≥ 2.0 was established for significance of a QTL.

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Results and discussion

Genetic variability for physical traits in almond

The phenotypic variability and the frequency distributions for the physical components of the almond kernel and nut are shown in Table 2 and Fig. 1 and 2. Most traits evaluated showed a normal distribution, although for some traits, such as nut T/L ratio and kernel width the distribution was skewed. The values of the parents were in the range of variability of the progeny, but for all the traits related to nut and kernel size (the three primary dimensions and weight), the parents' mean was away from the progeny mean. This deviation would be expected in traits subjected to constant breeding selection, as kernel size has been for a long time. Despite this deviation, some seedlings showed in all cases higher values than the best parent, thus opening up the possibility for improvement through breeding.

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Despite the similarity of the parents for many traits, this progeny showed a wide range of variability, although it cannot be compared with other populations. The only other genetic analysis of QTLs linked to the size of the almond nut and kernel (Sánchez-Pérez et al., 2007b) did not show the variability of the progeny.

Linkage map of QTLs controlling the physical components of the almond kernel and nut

The population studied was selected because of the wide range of variability of physical components of the nuts and kernels. A map from this population had already been published (Fernández i Martí et al. 2011) and was used for detecting QTLs controlling physical traits of the almond nuts and kernels. This map, previously constructed with 52 SSR markers, has been increased with 4 more SSRs, representing a total of 56 markers (Table 1). The position of these markers (Fig. 3) agrees with the last almond map published (Tavassolian et al. 2010). A LOD score of 2.0 was used to establish the presence of a QTL linked to the traits studied (width, thickness, length, weight, geometric diameter, spherical index, size, L/W, T/L and W/L). A total of 14 putative QTLs controlling these traits were detected in this analysis, corresponding to six genomic regions of the eight almond LGs. Only LG4 and LG8 did not show any QTL for almond nut and kernel traits. Some QTLs were clustered in the same region and/or shared the same molecular markers (Table 3). The LOD values for any given trait ranged from 2.06 to 5.17, explaining from 13.0 to 44.0% of the phenotypic variance of the trait.

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QTls for primary dimension (width, thickness and length)

Eight QTLs controlling the traits of nut width, thickness and length were detected in LG1, LG2, LG3, LG5, LG6 and LG7 (BPPCT020a, UDP98-025, BPPCT007, UDP96-008, CPSCT006, UDP98-412, CPPCT033 and PMS02) (Table 3, Fig. 3). The LOD of all traits studied ranged from 2.17 (PMS02) to 4.56 (BPPCT020a) and the percentage of phenotypic variance ranged from 15% (PMS02) to 30.6% (BPPCT020a).

In addition, eight QTLs were detected for the same kernel traits in LG1, LG3, LG5, LG6 and LG7 (CPPCT042, UDP96-008, BPPCT017, CPSCT006, BPPCT020b, UDP98-412, CPPCT039 and PMS02). Some of these QTLs shared the same locus for both nut and kernel (UDP96-008, CPSCT006, UDP98-412, and PMS02). The LOD of these three kernel traits ranged from 2.13 (PMS02) to 4.63 (UDP96-008). The percentage of phenotypic variance ranged from 13.5% (BPPCT020b) to 30.6% (UDP96-008). All LODs and percentages of variance explained are summarized in Table 2, giving the first information on QTLs linked to the primary dimensions of the almond nut and kernel. Nut and kernel primary dimensions are correlated, but not conclusively (Kester et al. 1993; Kodad and Socias i Company 2006). Our results coincide with this assertion as the same dimension for nut and kernel are not always controlled by the same QTL. Only 4 QTL are controlling the same dimension for nut and kernel. Nut and kernel width are linked to UDP96-008 marker, while nut and kernel length are linked to CPSCT006, UDP98-412 and PMS02 markers.

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QTLs for weight and size

Three QTLs were detected for nut weight and size at the beginning of LG1 (BPPCT020a; LOD of 2.47), LG2 (UDP98-025; LOD of 4.89), and LG7 (CPPCT033; LOD of 2.79). For kernel weight and size, the same QTL in LG7 (CPPCT033) was detected, but also a new QTL for weight in LG7 (CPSCT004) with a LOD of 2.90 (Table 3, Fig. 2). The total phenotypic variation for weight in nut and kernel was 44.6% and 14.4% respectively, whereas for size it was 30.4% and 16.2% respectively. Only Sánchez-Pérez et al. (2007b) had previously conducted nut weight examination, with two QTLs on LG1 and LG2 in the progeny 'R × D'. These two QTLs are located in the same position as ours, thus confirming the results. There is no previous information on QTLs linked to nut and kernel size. In other *Prunus* species very few studies have been carried out for fruit weight and size. In sweet cherry, Zhang et al. (2010) identified three QTLs linked to fruit size on LG2 and LG6 using SSR markers, whereas in peach, one QTL linked to fruit weight was detected on LG1 by Abbott et al. (1998) using RFLP, AFLP, RAPD and SSR markers.

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QTLs for derived dimensions (spherical index, geometric diameter, L/W, T/L and W/L ratios)

A total of ten QTLs were detected for spherical index, geometric diameter, and L/W, T/L, and W/L ratios, both in the nut and the kernel. Four QTLs were identified for the nut spherical index in LG2 (UDP98-025; LOD of 3.17), LG3 (BPPCT007; LOD of 2.17, and UDP96-008; LOD of 2.34), and LG7 (CPPCT033; LOD of 3.23) (Table 3, Fig. 3). Their total phenotypic variation was 69.4%. For the kernel spherical index only one QTL was identified on LG7 (CPPCT033; LOD of 2.80). Two QTLs were identified for nut geometric diameter, one on LG2 (UDP98-025; LOD of 2.71) and the other on LG6 (UDP98-412; LOD of 2.20). Two different QTLs were identified for the kernel geometric diameter, one on LG1 (BPPCT020a; LOD of 2.10) and the other on LG7 (CPPCT033; LOD of 3.12). Their total phenotypic variation for nut and kernel was 61.9%. These two traits are related to nut and kernel shape, which is a rather constant parameter despite the variation in size (Kodad and Socias i Company, 2006). However, only one QTL (CPPCT033) has been shown to be significant for the same trait (spherical index) in the nut and the kernel.

For the nut T/L ratio, three QTLs were located, one on LG1 (CPPCT042; LOD of 4.81), another on LG5 (CPSCT006; LOD of 3.57), and a third on LG7 (CPPCT033; LOD of 3.33). Their total phenotypic variation was 35, 22.3 and 23.7% respectively. The kernel T/L ratio was controlled by two QTLs, one located on LG1 (CPPCT042; LOD of 4.40), explaining a phenotypic variation of 20%, and another on LG2 (UDP96-013; LOD of 3.10), explaining 19.6% of the phenotypic variation (Table 2, Fig. 1). Four QTLs were identified for the nut and kernel L/W ratio, one on LG7 (CPPCT033; LOD of 2.13), one on LG2 (UDP96-013; LOD of 3.70), one on LG3 (UDP96-008; LOD of 5.17), and the last on LG6 (BPPCT020b; LOD of 2.58), explaining most of the phenotypic variation (R^2 of 76.7). Also four QTLs were located for the nut W/L ratio on three different linkage groups (LG1, LG5 and LG7). The nearest markers were BPPCT020a (LOD of 4.0) and CPPCT042 (LOD of 4.0) on LG1, CPSCT006 (LOD of 3.34) on LG5, and CPPCT033 (LOD of 3.45) on LG7, explaining a phenotypic variation of 96.5%. For the kernel W/L ratio, only one QTL was detected, CPPCT033 on LG7, with a LOD of 4.30 and explaining a phenotypic variation of 22.7%. However, only two QTLs have been shown to be significant for the same trait in the nut and the kernel. The CPPCT042 marker was linked to the T/L ratio for nut and kernel whereas the CPPCT033 marker was linked to the W/L ratio for nut and kernel.

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Correlations between the physical traits in almond

Table 4 shows the phenotypic correlations among the nut and kernel traits in almond. The three primary nut dimensions (length, width, thickness) were significantly correlated between them (0.88, 0.64, 0.52), as well as with weight (0.77, 0.89, 0.82), geometrical diameter (0.84, 0.93, 0.85), spherical index (0.61, 0.98, 0.95) and size (0.84, 0.93, 0.85). Weight showed positive and significant correlations with geometrical diameter (0.94), spherical index (0.89) and size (0.94). Also, geometrical diameter was correlated significantly with spherical index (0.94) and size (0.99). The derived ratios L/W, T/L and W/L were negatively correlated with length (-0.44, -0.73, -0.55), weight (-0.59), geometrical diameter (-0.51, -0.27) and size (-0.51, -0.27). The highest correlations for nut traits were found between width and spherical index (0.98), and between size and geometrical diameter (0.99). All these correlations were expected as involved in establishing the final size of the nut.

The three primary kernel dimensions were also positively and significantly correlated among them (0.35, 0.44, 0.42). Weight showed significant correlations with width (0.29), length (0.43), size (0.51), geometrical diameter (0.51) and spherical index (0.40), and size showed significant correlations with width (0.75), length (0.79), geometrical diameter (0.99) and spherical index (0.83). Also, width, thickness, and length of kernel showed positive and significant correlations with geometrical diameter (0.75, 0.79) and spherical index (0.75, 0.47, 0.32). The derived dimensions T/L and L/W were negatively correlated with length (-0.79, -0.34), width (-0.44, -0.84), geometrical diameter (-0.43, -0.40), spherical index (-0.30) and size (-0.43, -0.40) (Table 4). The highest correlations were found between size and geometric diameter ($r = 0.99$; $P\leq0.05$), between size and spherical index ($r = 0.83$; $P\leq0.01$) and between geometric diameter and spherical index ($r = 0.83$; $P\leq0.01$). As expected, the most important correlations were found between the same nut and kernel traits, as already reported by Kester et al (1993) and Kodad and Socias i Company (2006).

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Correlations between physical and chemical traits in almond

Almond quality is defined by both chemical and physical traits (Socias i Company et al. 2008). Consequently, both kinds of traits must be considered simultaneously because a breeding program may require the improvement of both physical and chemical traits (Kodad and Socias i Company 2006). Some chemical traits have already been considered from a breeding point of view, including the correlations among them, and showing significant correlations with physical traits (Kodad et al. 2006; Font i Forcada et al. 2011 and 2012). Table 5 shows the phenotypic correlations between chemical and physical traits in almond. The highest positive correlations found were between protein content and nut length (0.61), weight (0.51), geometrical diameter (0.52), and size (0.52). Oil content was highly and positively correlated with nut length (0.42) and size (0.38), and highly and negatively with geometrical diameter (-0.38) and spherical index (-0.30). The most significant and negative correlations were found between palmitic acid and nut thickness (-0.48), and the T/L (-0.52) and W/L (-0.46) ratios, as well as between stearic acid and nut width (-0.47). Positive correlations were also found between oleic acid and nut width (0.28), thickness (0.40), weight (0.24), spherical index (0.35), T/L (0.30), and W/L (0.29). Negative correlations were found between linoleic acid and nut width (-0.27), thickness (-0.38), weight (-0.25), spherical index (-0.33), T/L (-0.28), and W/L (-0.27). For the tocopherol homologues, only γ- and δ-tocopherol were negatively correlated with the nut L/W (-0.30, -0.34) and T/L (-0.32) ratios.

For the kernel traits, negative and significant correlations were found between thickness and α- (-0.29), γ- (-0.27), and δ-tocopherol (-0.30). All fatty acids (except oleic), oil and protein contents were negatively correlated with kernel width (-0.28, -0.26, -0.36, -0.32, -0.27, -0.34). Significant and positive correlations were found between kernel length and protein content (0.63), oil content (0.43), palmitic acid (0.25), stearic acid (0.38), and palmitoleic acid (0.41). Kernel weight and size were significantly correlated with protein content and oil (0.37, 0.36, 0.63, 0.40). For the other traits, the most significant and highest correlations were found between protein content and kernel length and geometric diameter (0.63).

Relationships of QTLs linked to chemical and physical traits in almond

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Correlations between the chemical and physical parameters controlling the same QTL were observed in five of the eight almond LGs (LG1, LG2, LG3, LG6 and LG7).

Two QTLs were detected on LG1, one close to the locus BPPCT020a and the other near CPPCT042. The traits controlled by these two loci were significantly correlated. For the first QTL, δ-tocopherol was negatively and significantly correlated with the nut T/L ratio (0.32). For the second QTL, stearic acid was negatively correlated with the nut T/L and W/L ratios (-0.35, -0.35), and positively correlated with kernel length (0.38). For the first QTL, δ-tocopherol was negatively and significantly correlated with the nut T/L ratio (-0.32).

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On LG2, one QTL near the locus UDP98-025 showed significant correlations with quality traits. Oleic acid was positively correlated with nut width (0.28), thickness (0.40), weight (0.24), and spherical index (0.35). No significant correlations were found for the nut geometric diameter and size. Linoleic acid showed negative correlations with nut width (-0.27), thickness (-0.38), weight (0.25), and spherical index (0.33), as expected because of its negative correlation with oleic acid (Font i Forcada et al. 2011).

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One QTL near the locus BPPCT007 was identified on LG3 controlling several traits. Palmitic acid was negatively correlated with nut width (-0.27) and spherical index (-0.37). Nut width was also positively and highly correlated with the nut spherical index (0.98, $P \leq 0.01$).

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On LG6, the QTLs close to the locus UDP98-412 correlated positively and significantly with stearic acid and nut and kernel length, as well as with total protein content and nut and kernel length. Additionally, a significant correlation was found between nut geometric diameter and protein content.

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On LG7 significant correlations were found between traits positioned near the marker CPPCT033. Negative correlations were found between δ-tocopherol and palmitic, stearic and linoleic acids, and positive with oleic acid (Font i Forcada et al. 2011). Negative but low correlations were found between δ-tocopherol and the nut L/W (0.34) and T/L ratios (0.32). No significant correlations were found between δ-tocopherol and the other traits. Positive and low correlations were found between oleic acid and nut thickness (0.40), spherical index (0.35), T/L (0.30), and W/L (0.29), as well as with kernel spherical index and W/L ratio (0.44). Negative and low correlations were found between linoleic, palmitic and palmitoleic acids with nut thickness, spherical index, and T/L and W/L ratios, as well as between these fatty acids and kernel W/L ratio (Table 5). A negative and significant correlation was found between stearic acid and kernel geometrical diameter (-0.27), and a positive correlation between stearic acid and kernel size (0.27). A negative correlation was found between linoleic acid and kernel spherical index (-0.27).

Despite the high number of correlations between physical and chemical traits in almond, the correlation between two different traits does not always match with the same QTL controlling these traits. This lack of coincidence shows that the two traits are genetically independent. Consequently, these trait types may be improved simultaneously, even if they are negatively correlated. Thus, the high complexity of an almond breeding program aiming at an addition of positive traits may be simplified with the help of this new knowledge.

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Conclusion

Fourteen QTLs associated with the physical traits of the almond nut and kernel were identified. At least one QTL was correlated with each trait with a significant probability ($P \leq 0.05$). Among these physical traits, nine (width, thickness, length, geometric diameter, size, spherical index, L/W, T/L and W/L) have been now studied for the first time in almond.

The results of this study, together with knowledge acquired of chemical components of the almond kernel, may allow a more sound almond breeding program, not only taking into account that quality is an increasing objective in almond breeding, but also that the physical and chemical traits may be improved simultaneously. The genetic information obtained after mapping these QTLs may be a very useful tool in attaining this breeding objective.

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Table 1 SSRs used for identification of QTLs in the almond cross 'Vivot' × 'Blanquerna'

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Species of origin	SSR name	Reference	No of SSRs tested	No of SSRs amplified	No of SSRs mapped	No of loci mapped	Percentage of total SSRs placed in the 'V × B' map
Peach	BPPCT	Dirlewanger et al. 2002	24	23	15	16	28
Peach	CPPCT	Aranzana et al. 2002	32	31	15	15	27
Jap. Plum	CPSCT	Mnejja et al. 2004	6	6	6	6	12
Almond	EPDCU	Howad et al. 2005	6	6	2	2	3
Peach	EPPCU	Howad et al. 2005	9	9	1	1	2
Peach	PCHGMS/Ma0	Sosinski et al. 2000; Yamamoto et al. 2002	5	5	2	2	4
Peach	UDP	Cipriani et al. 1999; Testolin et al. 2000	17	17	9	9	15
Cherry	Others	Cantini et al. 2001; Downey and Iezzoni 2000	11	9	6	6	9
-	Total	-	110	106	56	57	100

Table 2 Basic statistics for nut and kernel traits in the 'Vivot' × 'Blanquerna' almond mapping population.

Trait	Minimum	Maximum	Mean±SD
Nut			
Width (W), mm	13.89	26.87	20±2.4
Thickness (T), mm	10.85	17.39	14.36±1.2
Length (L), mm	19.84	39.68	27.53±3.5
Weight, g	1.21	7.2	3.48±0.9
Geometric diameter (GD), mm	997	5581	2711±132
Spherical index (SI)	50.2	149.9	96.7±9.5
Size, mm^3	2991	16743	8134±635
L/W	0.53	0.88	0.73±0.08
T/L	0.4	0.68	0.53±0.06
W/L	0.62	0.8	0.72±0.04
Kernel			
Width (W), mm	9.1	16	12.1±1.5
Thickness (T), mm	5.24	8.14	6.95±0.6
Length (L), mm	15.9	26.5	19.8±2.3
Weight, g	0.26	1.89	1.12±0.34
Geometric diameter (GD), mm	334.8	895.8	559.9±102
Spherical index (SI)	21	35.9	28.1±3.7
Size, mm^3	1005	2687	1679±347
L/W	0.43	0.74	0.62±0.07
T/L	0.25	0.5	0.36±0.05
W/L	0.36	0.79	0.58±0.09

Table 3 Putative QTLs identified in the 'Vivot' × 'Blanquerna' almond mapping population, the linkage group they mapped to, LOD score, closest marker and the percentage of phenotypic variance explained by each QTL.

	Trait	Abbreviations	LG	LOD	Up-Down Locus	% Exp
Nut	Width	Wn	2	3.02	UDP98-025	19.6
			3	2.73	BPPCT007	16.1
			3	3.02	UDP96-008	19.0
	Thickness	Tn	2	2.33	UDP98-025	16.0
			3	2.71	UDP96-008	21.7
			7	3.59	CPPCT033	23.0
	Length	Ln	1	4.56	BPPCT020a	30.5
			5	3.25	CPSCT006	18.9
			6	3.82	UDP98-412	23.0
			7	2.17	PMS02	15.0
	Weight	Wgn	1	2.47	BPPCT020a	16.8
			2	4.89	UDP98-025	27.8

Geometric Diameter	GDn	2	2.71	UDP98-025	16.7
		6	2.20	UDP98-412	13.5
Spherical Index	SIn	2	3.17	UDP98-025	20.6
		3	2.17	BPPCT007	13.2
		3	2.34	UDP96-008	14.6
		7	3.23	CPPCT033	21.0
Size	Sn	2	2.06	UDP98-025	15.0
		7	2.79	CPPCT033	15.4
L/W	L/Wn	7	2.13	CPPCT033	13.0
T/L	T/Ln	1	4.81	CPPCT042	35.0
		5	3.57	CPSCT006	22.3
		7	3.33	CPPCT033	23.7
W/L	W/Ln	1	4.00	BPPCT020a	21.2
		1	4.00	CPPCT042	30.8
		5	3.34	CPSCT006	20.7

			7	3.45	CPPCT033	23.8
Kernel	Width	Wk	3	4.63	UDP96-008	30.6
			5	2.44	BPPCT017	17.0
	Thickness	Tk	6	2.42	BPPCT020b	13.5
			6	2.86	UDP98-412	24.2
			7	3.02	CPPCT039	16.1
	Length	Lk	1	3.85	CPPCT042	27.2
			5	3.85	CPSCT006	16.8
			6	2.48	UDP98-412	23.6
			7	2.13	PMS02	16.4
	Weight	Wgk	7	2.90	CPCST004	14.4
	Geometric Diameter	GDk	1	2.10	BPPCT020a	13.1
			7	3.12	CPPCT033	18.6
	Spherical Index	SIk	7	2.80	CPPCT033	16.9
	Size	Sk	7	3.19	CPPCT033	16.2

L/W	L/Wk	2	3.70	UDP96-013	19.7
		3	5.17	UDP96-008	44.0
		6	2.58	BPPCT020b	13.0
T/L	T/Lk	1	4.40	CPPCT042	30.0
		2	3.10	UDP96-013	19.6
W/L	W/Lk	7	4.30	CPPCT033	22.7

Table 4 Phenotypic correlations between pairs of almond nut and kernel physical traits in almond

Trait	Wn	Tn	Ln	Wgn	GDn	SIn	Sn	LWn	TLn	WLn	Wk	Tk	Lk	Wgk	GDk	SIk	Sk	LWk	TLk	WLk
Wn	-	**0.88****	**0.64****	**0.89****	**0.93****	**0.98****	**0.93****	**-0.63****	-0.04	**0.28***	**0.95****	**-0.33****	**0.45****	**0.31***	**0.68****	**0.63****	**0.68****	**-0.86****	**-0.51****	**0.48***
Tn		-	**0.52****	**0.82****	**0.85****	**0.95****	**0.85****	-0.18	-0.20	**0.31***	**0.85****	-0.15	**0.34****	**0.30***	**0.63****	**0.67****	**0.63****	**-0.70****	**-0.32****	**0.51****
Ln			-	**0.77****	**0.84****	**0.61****	**0.84****	**-0.44***	**-0.73****	**-0.55****	**0.57****	0.11	**0.93****	**0.41****	**0.78****	**0.36****	**0.78****	**-0.49****	**-0.81****	**-0.31***
Wgn				-	**0.94****	**0.89****	**0.94****	**-0.59****	-0.02	0.01	**0.83****	**0.31****	**0.59****	**0.47****	**0.71***	**0.54****	**0.71****	**-0.75****	**-0.60****	0.24
GDn					-	**0.94****	**0.99****	**-0.51****	**-0.27***	-0.03	**0.88****	**0.29***	**0.68****	**0.36****	**0.80****	**0.61****	**0.80****	**-0.76****	**-0.64****	0.22
SIf						-	**0.94****	**-0.46****	0.05	**0.30***	**0.93****	**-0.27***	**0.42****	**0.30****	**0.69****	**0.66****	**0.68****	**-0.81****	**-0.45****	**0.50****
Sn							-	**-0.51****	**-0.27***	-0.03	**0.88****	**-0.28***	**0.68****	**0.36****	**0.80****	**0.61****	**0.80****	**-0.76****	**-0.64****	0.21
LWn								-	**0.39****	-0.10	**-0.55****	**0.44****	**-0.36****	0.14	**-0.35****	0.20	**-0.35****	**0.64****	**0.54****	-0.16
TLn									-	**0.88****	0.12	0.18	**-0.79****	0.22	**-0.36****	0.14	**-0.36****	0.03	**0.71****	**0.77****
WLn										-	**0.32****	0.03	**-0.66****	0.01	-0.20	**0.26***	-0.21	**-0.30****	**0.48****	**0.91****
Wk											-	**0.35***	**0.44****	**0.29***	**0.75****	**0.75****	**0.75****	**-0.84****	**-0.44****	**0.55****
Tk												-	**0.42***	0.21	0.22	**0.47****	0.22	**0.70****	**0.67****	-0.09
Lk													-	**0.43****	**0.79****	**0.32***	**0.79***	**-0.34****	**-0.79****	**-0.50****
Wgk														-	**0.51****	**0.40****	**0.51****	-0.10	-0.19	-0.11
GDk															-	**0.83****	**0.99***	**-0.40****	**-0.43****	0.002
SIk																-	**0.83****	**-0.30***	0.07	**0.44****
Sk																	-	**-0.40****	**-0.43****	0.002
LWk																		-	**0.65****	**-0.48****
TLk																			-	**0.34****
WLk																				-

Correlations in bold are significant at *$P \leq 0.05$ and **$P \leq 0.01$

Table 5 Phenotypic correlations between chemical and physical traits of almond nut and kernel

Trait[z]	Wn	Tn	Ln	Wgn	GDn	SIn	Sn	LWn	TLn	WLn	Wk	Tk	Lk	Wgk	GDk	SIk	Sk	LWk	TLk	WLk
Protein content	**-0.34****	**-0.38****	**0.61****	**0.51****	**0.52****	**-0.39****	**0.52****	-0.15	**-0.39****	**-0.34****	**-0.34***	0.12	**0.63****	**0.37****	**0.63****	**0.40***	**0.63****	-0.18	**-0.39****	**-0.26***
Oil content	**-0.29***	**-0.25***	**0.42****	**0.29***	**-0.38****	**-0.30***	**0.38****	0.16	**0.26***	0.19	**-0.27***	0.34	**0.43****	**0.36****	**-0.40***	-0.21	**0.40****	0.19	**0.33***	0.14
Oleic acid	**0.28***	**0.40****	0.02	**0.24***	0.25	**0.35****	0.25	0.06	**0.30***	**0.29***	**0.29***	0.03	0.03	0.03	0.16	**0.27***	0.16	0.18	0.07	**0.31***
Linoleic acid	**-0.27***	**-0.38***	-0.02	**-0.25***	-0.23	**-0.33****	-0.23	-0.05	**-0.28***	**-0.27***	**-0.28***	-0.04	-0.01	-0.01	-0.17	**-0.27***	-0.17	-0.17	-0.05	**-0.30***
Palmitic acid	**-0.27***	**-0.48****	-0.13	**0.27***	-0.19	**-0.37****	-0.19	-0.18	**-0.52****	**-0.46****	**-0.26***	-0.005	**0.25***	0.20	-0.01	-0.23	-0.01	0.18	-0.22	**-0.50***
Stearic acid	**-0.47***	-0.05	**0.37***	-0.22	-0.24	-0.08	-0.24	0.04	**-0.35****	**-0.35****	**-0.36***	-0.60	**0.38***	0.11	**-0.27***	-0.08	**0.27***	-0.03	0.21	**-0.30***
Palmitoleic acid	-0.25	**-0.31***	**0.28***	-0.16	-0.09	**-0.30***	-0.09	-0.004	**-0.57****	**-0.62***	**-0.32***	0.10	**0.41****	0.02	0.09	-0.23	0.09	**0.28***	**-0.27***	**-0.49****
α-tocopherol	0.22	0.12	0.11	0.21	0.09	0.18	0.19	-0.24	-0.02	0.10	0.15	**-0.29***	-0.01	-0.01	-0.03	-0.06	-0.03	**-0.25***	-0.15	0.14
γ-tocopherol	0.10	-0.07	0.11	0.08	0.09	0.04	0.09	**-0.30***	-0.18	-0.04	0.05	**-0.27***	0.02	0.02	-0.08	-0.14	-0.09	-0.15	-0.17	0.02
δ-tocopherol	0.07	-0.14	0.18	0.06	0.19	-0.002	0.09	**-0.34****	**-0.32***	-0.17	0.05	**-0.30***	0.15	0.15	0.05	-0.06	0.05	-0.08	-0.21	-0.12

Correlations in bold are significant at *$P \leq 0.05$ and **$P \leq 0.01$

[z] The units for the chemical components are (Font i Forcada et al., 2011): protein and oil contents as % of dry weight; fatty acids as % of the total oil content; tocopherol homologues as $mg \cdot kg^{-1}$ of oil.

Figure captions

Fig. 1 Frequency distribution of nut physical traits in the ‘V×B’ population. Values for parents are indicated by arrows (⇩Vivot; ⬇ Blanquerna).

Fig. 2 Frequency distribution of kernel physical traits in the ‘V×B’ population. Values for parents are indicated by arrows (⇩Vivot; ⬇ Blanquerna).

Fig. 3 Combined linkage map of ‘Vivot’ × ‘Blanquerna’ population constructed using MAPCHART v. 2.1 (Voorrips, 2000) showing putative QTLs associated with physical nut and kernel traits in almond.