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Inheritance of shell and kernel shape in almond (*Prunus dulcis*)

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ABSTRACT

Shape of almond kernel has an important impact in the final commercial value. Elliptic shapes are more common while round shapes, 'Marcona' types, are scarce and very appreciated. In order to increase the efficiency of the breeding programs for specific shape is important to know the genetic control of this trait. In this work, heritability of length, width, thickness, roundness and globosity, of shell and kernel, was estimated by midparent-offspring regression (narrow sense, h^2) and by variance components analysis (broad sense, H^2) for three years, using full-sib and half-sib offsprings coming from four crosses designed only for this objective. Length and roundness of shell and kernel showed the highest heritability by both methods every year, being intermediate or very low for the other traits. Kernels were usually less rounded than their shells. Values near one of the ratio h^2/H^2 were observed for length and roundness, showing an absence of non-additive effects for these two traits. In general, a normal phenotypic distribution was observed for all the traits and certain transgressive segregation was observed in the descendants. The results confirmed the complex architecture of this trait transmitted quantitatively, where additive effects play the mayor role.

1. Introduction

Almond [*Prunus dulcis* (Miller) D.A. Webb] is a very important nut crop whose importance ranks first in world nut production, with 1.07 million tons of kernel basis (Almond Board of Australia (ABA), 2016). Nut and kernel shape traits are involved in almond quality (Gradziel et al., 2013) and its shape must meet the different commercial requirements of the industry, which depend on destination (fresh, roast, fried, nougat, etc), and consumers.

In general, almond nut and kernels have amygdaloidal shape (from which comes the Latin name *Amygdalus*). However, among commercial cultivars and especially in offsprings generated in the breeding programs, shapes are endless. Due to commercial constraints or perhaps because it is the least common, there is a special interest in rounded kernels. In the past, global kernels were previously preferred for sugared almonds (drageés) because only a thin layer of sugar was needed to cover them (Socias i Company et al., 2008). At the present, as sugar become less expensive and almond meal more expensive, flatter kernels are preferred because they allow a thicker sugar layer. For laminates types, elongated shapes are preferable. In addition, very globular shapes are not desirable for gourmet chocolates, since they can leave much out of the tablet, being preferred flat shapes. A clear example is the well-recognized and appreciated 'Marcona-type', per example in making "Alicante" nougat, being the most economically valued. Due to that, producers frequently sell other cultivars of quite similar shape, such as 'Carreró' or 'Antoñeta', as 'Marcona'.

Depending on the cultivar and the region, about 12 weeks after flowering the whole fruit had reached full size and afterwards the embryo increased its size with a concurrent decrease of the endosperm (Hawker and Buttrose, 1980; Martínez-Gómez et al., 2008). It is evident that the shell will determine in a high measurement the final shape of the kernel which will be developed inside. Clearly, the mesocarp, endocarp and the kernel will have a similar shape, but with some variability. In summary, seems that the mother tree determines the shape of all the nuts of the tree, regardless of the pollinator.

Also chemical traits seem to be controlled by the female progenitor, with independence of the pollinizer. The absent of the pollinizer effect in some characters of the shell and the kernel has been demonstrated before (Dicenta and García, 1993; Kumar and Das, 1996; Dicenta et al., 2000). The effect of the pollinator, that is the effect of its pollen, on the characteristics of the endosperm and the embryo, is called "xenia", while the effect on the surrounding tissues of maternal, mesocarp and endocarp, origin is called "metaxenia" (Swingle, 1928). Xenia or metaxenia have been reported in different fruit tree species such as pear and apple (Denney, 1992). In the case of almond, the influence of pollinators on flowering, production and nut traits (Dicenta and García, 1993), kernel flavour (Dicenta et al., 2000), kernel amygdalin content (Arrázola, 2002), ripening time and fruit size (Kumar and Das, 1996),

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chilling requirements for seed germination (Kester et al., 1977; García-Gusano et al., 2010) has been studied. The results of most of these studies showed no influence of pollinators on the characteristics examined.

The genetic improvement to meet different kernel market needs is carried out for breeders in almond breeding programs around the world. Knowing the degree of heritability (narrow-sense) of quantitative traits, such as nut shape traits, is very helpful to design an efficient breeding strategy (Iwanami et al., 2008), and to estimate the gain to be expected under mass selection.

A complex genetic control for nut and kernel shape traits such as width, thickness or length of almond has been reported (Fernández i Martí et al., 2013). In addition, heritability and other genetics components were studied for nut traits in almond using different populations (Spiegel-Roy and Kochba, 1981; Dicenta et al., 1993; García et al., 1994; Sánchez-Pérez et al., 2007; Martínez-García et al., 2014). In some of these studies, progenies presented phenotypes outside of the phenotypic range of the parental populations from which they were derived (Sánchez-Pérez et al., 2007; Martínez-García et al., 2014), the generation of these extreme phenotypes is called transgressive segregation (Rieseberg et al., 1999), a phenomenon very useful for breeders.

The main objective of this work was to study the inheritance of shell and kernel shape traits in almond and estimate their heritability, using a well design experiment with four cultivars phenotypically different for these traits and their progenies for three years, in order to design efficient strategies of breeding for specific fruit shape in almond.

2. Material and methods

2.1. Plant material

Four different crosses between parents that present rounded (R) kernels ('Marcona' and 'Del Cid') and elongated (L) kernels ('Peraleja' and 'Desmayo Largueta') were developed by hand pollination. Pollen of male progenitors was collected and dried at room temperature for 2 days. Then it was stored at 4 °C till pollinations. Flowers at "E" stage (just before opening) of female progenitors were emasculated and hand-pollinated using a paintbrush. According to the shape of progenitors, the following progenies were obtained: 'Marcona' × 'Del Cid' (R × R), 'Marcona' × 'Peraleja' (R × L), 'Peraleja' × 'Marcona' (L × R), and 'Peraleja' × 'Desmayo Largueta' (L × L). About 150 flowers were pollinated for each cross. We assumed that the selected parents are unrelated without a common ancestor, which can ensure that the inbreeding coefficient was zero (Falconer, 1960). The cultivars and their studied progenies were grown in the experimental field of CSIC in Santomera, Murcia (SE of Spain), with fertirrigation. The final number of seedlings obtained and planted by family was 30.

Four years later, when trees came into bearing, at maturity time (when > 90% of mesocarps were opened), five mature fruits from parents and seedlings were randomly picked up from the trees by hand, their mesocarp removed and the nuts stored at room temperature for two months. For each nut, shell length (mm), width (mm) and thickness (mm) were measured. Afterwards, the nuts were cracked using a hammer and kernel length (mm), width (mm) and thickness (mm) were recorded. Finally, roundness (length/width) and globosity (width/thickness) for the shell and the kernel were calculated (Fig. 1). Finally, to determine the differences between the shape of shells and kernels, a comparison between roundness and globosity of shell and kernel was realized.

The process was repeated for three consecutive years, for both the parents and the descendants of each cross. We have used the following terms: Long vs. Short, Wide vs. Narrow, Thick vs. Thin, Rounded vs. Elongated and Globular *vs.* Flat, to define the different shapes of shells and kernels (Fig. 1).

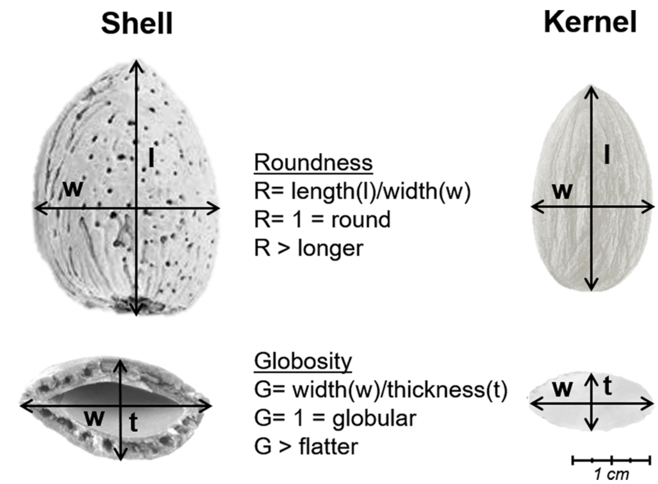

Fig. 1. Almond shell and kernel measurements. Length (l), width (w), thickness (t), Roundness (R) and Globosity (G).

2.2. Data analysis

Since our experiment was carried out under field conditions, environmental factors generated an unequal number of data (missing values) for each cross and each year. In this sense to obtain a more precise estimates, instead of the usual sample mean, least-squares means (Harvey, 1960; Searle et al., 1980) were calculated, by the R package lsmeans (Lenth, 2016). Differences between genitors, families and years for each trait were obtained by Tukey HSD (Tukey Honest Significant Differences) test (Tukey, 1949), using the R package lsmeans (Lenth, 2016).

Two different methods were used to estimate the heritability of traits studied. Firstly, heritability was estimated by components of variance between and within families. The variance between families (Vb) and variance within families (Vw) were calculated using one-way Anova. We used the formula $H^2 = (2 \times Vb)/(Vb + Vw)$ to calculate the broad sense heritability (Kearsey, 1965; Mather and Jinks, 1982; Dicenta et al., 1993). According to Kearsey (1965), we assumed our Vb a compound of dominance and additive measure, which make this estimation a good approximation to the broad sense heritability (H^2). The narrow sense heritability (h^2) was calculated by midparent-offspring regression (Falconer, 1960), where the regression coefficient "b" is the heritability in narrow sense.

In our experiment, where estimates of heritability are based in the same genotypes grown in the same environment, the ratio h^2/H^2 can detect the presence of dominance. Values of h^2/H^2 near one imply the absence of non-additive effects.

Finally, histograms of frequency for roundness and globosity of shell and kernel of families with data of all years, were obtained. In the case of unclear patterns and to check unimodality of our distributions, a dip test of unimodality (Hartigan and Hartigan, 1985) has been carried, using the R package diptest (Maechler, 2016).

3. Results

3.1. Shell traits of progenitors

Regarding shell, the cultivar with the longest shell was 'Desmayo Largueta' (34.89 mm) and 'Del Cid' was the cultivar with the shortest (26.39 mm) (Table 1). In general, 'Marcona' was the cultivar with the widest shells (23.77 mm) and 'Peraleja' the cultivar with narrowest (19.21 mm). For thickness, 'Marcona' was the cultivar with the thickest shell (17.83 mm) and 'Peraleja' the cultivar with the thinnest (13.79 mm). The values found in the progenitors were those expected for each cultivar, although some variability was observed between