Transfer of a high-seal but high crack-out shell structure from Prunus webbii to cultivated almond

Gradziel T.M.

in

Zakynthinos G. (ed.).
XIV GREMPA Meeting on Pistachios and Almonds

Zaragoza : CIHEAM / FAO / AUA / TEI Kalamatas / NAGREF
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 94

2010
pages 229-233

To cite this article / Pour citer cet article

Gradziel T.M. **Transfer of a high-seal but high crack-out shell structure from Prunus webbii to cultivated almond.** In : Zakynthinos G. (ed.). *XIV GREMPA Meeting on Pistachios and Almonds.* Zaragoza : CIHEAM / FAO / AUA / TEI Kalamatas / NAGREF, 2010. p. 229-233 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 94)

Transfer of a high-seal but high crack-out shell structure from *Prunus webbii* to cultivated almond

T.M. Gradziel

Department of Plant Sciences, University of California, Davis, California 95616 (USA)

Abstract. A novel shell structure has been transferred from *Prunus webbii* to cultivated almond through a series of recurrent backcrosses where the trait appears dominant in progeny. Resulting selections possess good nut quality with a kernel mass of up to 1 g or more. The shell is composed of a single highly lignified inner endocarp rather than inner plus outer lignified layers enclosing a middle layer containing vascular bundles, as found in most cultivated almonds. In these novel shell types, the vascular bundles are peripheral to the highly lignified inner endocarp but often directly adjacent to the outer fleshy mesocarp or hull. Shell thickness can be as thin as 1 mm in many areas resulting in high (kernel meat / kernel meat plus shell) crack-out ratios of 0.70 or more. Despite the high crack-out ratios, shell seal integrity can range from moderate to high depending on individual genotype. High shell seal integrity results from both the high lignin density found in fully developed shells as well a modified endocarp development pattern which appears to avoid internal structural stresses which can result in shell fractures in cultivated almond types. The developmental and structural differences of these novel shell types thus have potential both for breeding improved varieties as well as advancing our basic understanding of kernel, shell and hull development.

Keywords. Shell structure breeding – *Prunus webbii.*

Transfert d'une structure de coque fortement scellé mais à « crack-out » élevé de Prunus webbii _à l'amandier cultivé_

Résumé. *Une nouvelle structure de coque a été transférée de* Prunus webbii *aux amandiers cultivés à travers une série de rétrocroisements périodiques où cette caractéristique apparaît sous forme dominante dans la descendance. Les sélections résultantes possèdent des fruits de bonne qualité avec une masse d'amande maximale de 1 g ou plus. La coque est composée d'un seul endocarpe intérieur très lignifié plutôt que de couches intérieure plus extérieure lignifiées renfermant une couche intermédiaire contenant les faisceaux vasculaires, comme chez la plupart des amandiers cultivés. Dans ces nouveaux types de coque, les faisceaux vasculaires sont périphériques à l'endocarpe intérieur très lignifié mais souvent directement adjacent au mésocarpe extérieur charnu ou cosse. L'épaisseur de la coque peut montrer une épaisseur de seulement 1 mm dans de nombreuses zones, ce qui donne un fort ratio de "crack-out" (masse de l'amandon / masse de l'amandon plus coque) de 0,70 ou plus. Malgré un ratio de "crack-out" élevé , l'intégrité de jointure de la coque peut aller de modérée à élevée en fonction de chaque génotype. Une forte intégrité de jointure de la coque résulte à la fois de la haute densité de lignine trouvée dans les coques entièrement développées ainsi que d'une modification du modèle de développement de l'endocarpe qui semble éviter les contraintes structurelles internes pouvant entraîner des fractures de coquille chez les types d'amandiers cultivés. Les différences structurelles et de développement de ces nouveaux types de coques ont donc un potentiel pour la reproduction des variétés améliorées, ainsi que pour faire avancer notre compréhension de base du développement de l'amandon, de la coque et de la cosse.*

Mots-clés. *Amélioration génétique – Structure de coque –* Prunus webii.

I – Introduction

The lignified shell or endocarp of almond (*Prunus dulcis*) serves many important biological and commercial roles. Under natural conditions it helps protect the almond kernel from damage, including animal and bird feeding as well as insect pests and diseases. The intact shell also protects the inner kernel from desiccation while at the same time facilitating water uptake during germination. Well-sealed and highly lignified shells are generally considered to give the best protection to enclosed kernels. High shell mass and/or degree of lignification can cause problems during processing, as it often makes the nuts more difficult to shell and may result in fragments of the shell being embedded in the kernel. Thick or dense shells also reduce the kernel/nut cracked-out ratio and so economic return. The shells of cultivated almond are distinct from those of many of its closely related species since it is composed of an inner and outer lignified layer enclosing an only partially lignified central layer containing an intricate netting of vascular bundles. These vascular bundles, which grow along the surface of the inner endocarp, feed vascular strands which radiate outward through constricting openings or pores in the outer lignified endocarp to become the fruit mesocarp or flesh. In many related species, including *Prunus webbii*, *P. argentia*, and peach, (*P. persica*), the shell consists only of a highly lignified inner layer enclosed by the netted vascular bundles from which secondary strands radiating directly as a mesocarp or flesh tissue. The transfer from *P. webbii* to cultivated almond of a single layered, peach-type endocarp is described. In addition to the recovery of novel and commercially useful shell types, this work has advanced our understanding of the mechanisms of shell development and breakdown in cultivated almond.

II – Materials and methods

Controlled crosses. Trees of *P. webbii*, grown from seed collected in Yugoslavia, were used as the pollen parent in a cross with the California almond variety Mission (syn. Texas). Progeny were sib mated, with F_2 seedlings grown out at the Wolfskill Experimental Orchard, Winters, California. Individuals demonstrating good kernel size and quality as well as a P. webbii-type shell structure were selected for a series of backcrosses, initially to the variety Ferragnes and subsequently to the variety Nonpareil.

Nut evaluation. Evaluations were made on mature, dry almond fruit from which the hulls had been removed by hand. Endocarp type was determined by using parental types (Fig. 1) as references. Shell fracturing was determined by examining the surface of the nuts before and after harvest. The site of shell fractures was determined by examining longitudinal cross-sections of mature nuts, occasionally using a dilute sodium hypochlorite solution to remove soft tissue.

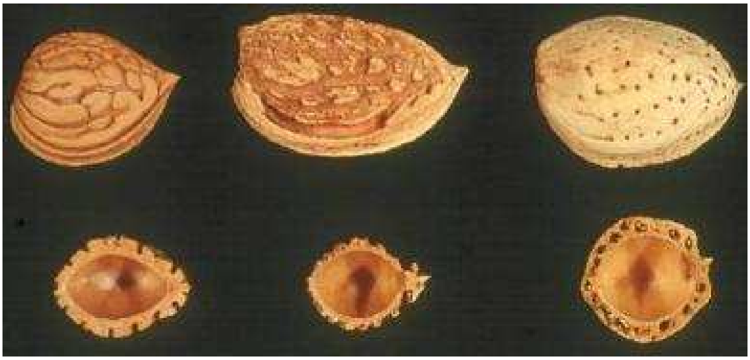

Fig. 1. Endocarp phenotypes of Mission almond (right), Mission × *P. webbii* hybrid (left) and F_2 progeny (center).

III – Results and discussion

The interspecific cross between the cultivated almond variety Mission to *P. webbii* resulted in progeny which uniformly demonstrated highly lignified *P. webbii*-type shells (see Fig. 1) yet were intermediate in size between the two parents. Sib mating among these individuals often produced F_2 progeny demonstrating the range of shell types between parental types, with most seedling trees producing the multi-layer softer shell typical of cultivated almond. One individual, 7914-26, was selected for backcrossing-based on a highly sealed, *P. webbii* inner endocarp with only traces a lignified outer endocarp (Fig. 1). The more friable nature of the outer endocarp would sometimes leave traces of outer endocarp and associated vascular bundles remaining with the hulls following their removal. Back crossing 7914-26 as pollen parent to the hard-shelled variety Ferragnes resulted in progeny populations in which the single layer *P. webbii*-type shell phenotype predominated (Fig. 3) and where the degree of lignification in the inner endocarp layer sometime approached that of the of the F_1 parent. Several BC_1 selections demonstrated acceptable commercial kernel size combined with the thin, highly lignified and single layered endocarp similar to the F_2 parent. Further backcrosses of BC_1 selections to the cultivated almond variety Nonpareil again produced populations of varying shell types, but where the single layer, *P. webbii*-type shell phenotype predominated. The dominant expression of the *P. webbii*-type shell allowed the selection of several BC_2 progeny combining good commercial kernel quality and highly sealed shells (Figs 4 and 5). The crack-out ratio (kernel mass/kernel plus shell mass) of BC_2 selections consistently exceeded 60% and was higher than 75% in some individuals (Fig. 5).

Fig. 2. Typical endocarp phenotypes in (Mission × *P. webbii*) F_2 progeny.

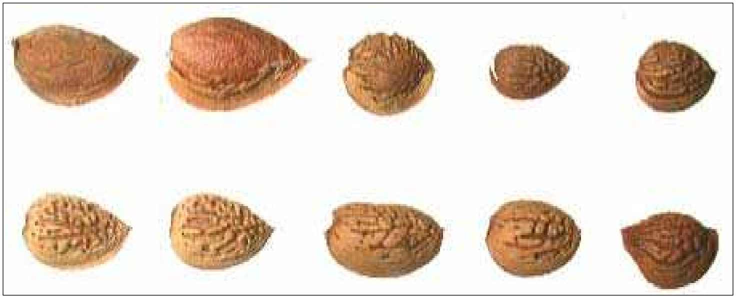

Fig. 3. Representative endocarp phenotypes in BC_1 backcross to Ferragnes.

Fig. 4. Advanced selection from BC_2 to Nonpareil showing good kernel and nut quality (Nonpareil and Carmel kernels shown in inset for comparison).

Fig. 5. Advanced selection from BC_2 to Nonpareil showing good shell-seal with the high crackout of 77%.

When shell splitting occurred in the *Nonpareil* backcross parent, the site of fracture was rarely at the endocarp suture line as often occurs in peach, but usually was located adjacent to the suture along the vascular bundle channel that feeds the funiculas to the abortive ovule (Table 1). (In almond flowers, two ovules are initially formed, one on either side of the suture line, though, typically, only one ovule develops fully because of strong selection against commercially undesirable double kernels). Shell fractures of this type can even occur in hard-shelled varieties such as Marcona (Fig. 6) though the separate fractures at both the inner and outer endocarp make it difficult to evaluate shell seal integrity without careful nut dissection. The strong association of inner endocarp fracturing adjacent to the vascular bundles feeding the abortive ovule suggests that the degeneration of this tissue predisposes the adjacent endocarp layers subsequent failure. The highly-sealed, F_2, BC_1 and BC_2 selections appeared to avoid this structural "Achilles-heel" affect, perhaps because of a greater physical and or developmental separation between the inner endocarp and vascular bundles in these genotypes.

Table 1. Proportion (%) and site of fractured endocarps of Nonpareil almonds collected before and after harvest by mechanical tree shaker

	Site of shell fracture		
	Suture	**Developing ovule**	**Abortive ovule**
Before	0.00	4.28	19.49
After	0.63	15.53	60.06

Fig. 6. Marcona almond shell showing fractures at the inner and outer endocarp layers associated with the site of abortive ovule vascular strand.

In progeny phenotypes of these select crosses, the high crack-out *P. webbii*-type endocarp dominated when either a hard-type shell (Ferragnes) or soft-type shell parent (Nonpareil) was used. Shell hardness is controlled by the *D/d*-locus where *D*- shells are hard and *dd* are soft (Kester *et al.*, 1977; Spiegel-Roy and Kochba, 1981; Dicenta *et al.*, 1993). In addition to the final degree of lignification, these genotypes also differ in their development time. *D*- genotypes become lignified at the beginning of Stage II while in *dd* genotypes, lignification does not occur until after completion of Stage II, leaving these genotypes vulnerable to earlier insect feeding on the developing nuts (Grasselly, 1972; Kester and Gradziel, 1996). Within this traditional germplasm, the timing of shell hardening has been positively correlated to the final shelling percentage (Kester and Gradziel, 1996). Consequently, the *P. webbii*-type endocarp, because it appears to primarily suppress outer endocarp layer development, may not only facilitate the breeding of high-sealed, high crack out almond cultivars, but when paired with the high lignin *D*-allele may promote earlier shell lignification and so greater resistance to insects which feed on the early developing fruit.

References

Dicenta F., García J.E. and Carbonell E., 1993. Heritability of fruit characters in almond. In: *J. Horti. Sci.*, 68, p. 121-126.

Grasselly C., 1972. L'amandier; caractères morphologiques et physiologiques des variétés, modalité de leurs transmissions chez les hybrides de première génération. University of Bordeaux.

Kester D.E. and Gradziel T.M., 1996. Almonds (*Prunus*). In: Moore J.N. and Janick J. (eds). *Fruit Breeding*. New York: Wiley, p. 1-97.

Kester D.E., Hansche P.E., Beres W. and Asay R.N., 1977. Variance components and heritability of nut and kernel traits in almond. In: *J. Amer. Soc. Hort. Sci.*, 102, p. 264-266.

Spiegel-Roy P. and Kochba J., 1981. Inheritance of nut and kernel traits in almond (*Prunus amygdalus* Batsch). In: *Euphytica*, 30, p. 167-174.