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Influence of stratification, heat and removal of teguments on breaking of seed dormancy in almond

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SUMMARY – In this work, the effect of stratification, heat, and endocarp and tegument removal on the breaking of seed dormancy was studied in 3 almond [*Prunus dulcis* (Mill.) D.A. Webb] cultivars ('Desmayo Largueta', 'Del Cid' and 'Wawona') with a broad range of flowering times. Two different trials were carried out. In the first one, the nuts with endocarp were stratified at 7ºC for 15 weeks. In the second, the nuts with endocarp were stratified at 7ºC for 4 weeks. Then, non-germinated seeds were kept at 22ºC for 7 weeks more to promote germination. After this time, the remaining non-germinated nuts were cracked and the tegument was removed. The percentage of germinated seeds of each cultivar was recorded weekly in both assays. The results showed a different effect of each treatment on seed germination for each cultivar, related to their flowering time. The heat treatment did not induce germination in seeds that had not fulfilled their chilling requirements. The removal of the endocarp and the tegument in seeds that had not fulfilled their chilling requirements, produced the same effect as stratification and, finally, similar percentages of seed germination were observed in comparison with the first treatment.

Key words: Almond, *Prunus dulcis*, germination, seed dormancy, tegument, endocarp, stratification requirements, heat requirements.

RESUME – *"Influence de la stratification, de la chaleur, et de l'élimination des téguments sur la rupture de dormance des semis d'amandier". Au cours de ce travail nous avons étudié l'effet de la stratification, de la chaleur, et de l'élimination de l'endocarpe et du tégument dans la rupture de la dormance de 3 variétés ('Desmayo Largueta', 'Del Cid' et 'Wawona') d'amandier [*P. dulcis *(Mill.) D.A. Webb] avec des dates de floraison distinctes. Dans le premier traitement, les semis avec endocarpe ont été stratifiés pendant 15 semaines à 7ºC. Dans le second traitement, les semis avec endocarpe ont été stratifiés à 7ºC pendant 4 semaines. Les semis qui n'ont pas germé ont été exposés à 22ºC pendant 7 semaines et après cette exposition l'endocarpe et le tégument ont été supprimés des semis qui n'ont pas germé. Chaque semaine le pourcentage de germination des semis a été calculé. Les résultats ont mis en évidence une relation entre les besoins de stratification des semis et la date de floraison de chaque variété. Le traitement à la chaleur ne peut pas induire la germination des semis qui n´ont pas satisfait leurs besoins en froid. L'élimination de l'endocarpe et du tégument produit une augmentation de la germination similaire à l´effet de la stratification de telle façon que les pourcentages finaux de germination sont similaires dans les deux traitements.*

Mots-clés : *Amandier,* Prunus dulcis*, germination, dormance des semis, tégument, endocarpe, stratification, chaleur.*

Introduction

Seed dormancy is an adaptive mechanism that protects many plant species from freezing damage during the winter. This phenomenon affects seed germination and later seedling growth (Lipe and Crane, 1966). Seeds from almond and other Rosaceae species require a cold treatment under humid conditions to overcome the dormancy and promote their germination. Temperature is the main factor affecting the germination rate. Temperatures between 2ºC and 7ºC are generally the most efficient. Abbot (1955) indicated –5ºC as the least effective temperature for seed germination. In addition, temperatures higher than 17ºC could re-impose the seed dormancy.

In almond nuts, the endocarp has a mechanical effect, affecting gas exchange and preventing seed imbibition and washing-out of the tegument hormones, thus modifying the stratification period required for germination (Du Toit *et al.*, 1979). In addition, tegument removal has been described as a mechanism to accelerate seed germination (Chao and Walker, 1966; Zigas and Coombe, 1977).

In this study, the effect of stratification, heat, and endocarp and teguments removal on the breaking of seed dormancy was studied in 3 almond cultivars with a broad range of flowering time.

Materials and methods

The almond cultivars studied were the Spanish 'Desmayo Largueta' (early-flowering and hard-shelled) and 'Del Cid' (middle-flowering and hard-shelled) and the American 'Wawona' (late-flowering and soft-shelled). These cultivars cover a broad range of flowering times within the almond species.

Samples of 30 mature nuts from open-pollination of the above mentioned almond cultivars were treated in a 2% TMTD® (Tetramethylthiuram Disulfide) fungicide solution for 30 minutes. Later, the samples were immersed in water for 48 hours, and placed in darkness inside plastic mesh bags in humid vermiculite. Seed germination was defined by the emergence of the radicle. Two different treatments were applied to seeds with endocarp. In the first treatment, nuts were stratified at 7ºC for 15 weeks following the classical stratification method. In the second treatment, nuts were stratified at 7ºC for 4 weeks. Then, the non-germinated seeds were exposed to 22ºC for 7 weeks to promote germination. After this time, the remaining non-germinated nuts were cracked and the tegument was removed.

The number of germinated seeds of each cultivar was recorded weekly for both treatments, and later the percentage of germinated seeds was calculated.

Results and discussion

Figure 1 shows the results for the first treatment as accumulated percentages of seed germination each week for the three studied cultivars. As can be observed, seed germination started the fifth week of the treatment for the cultivars 'Desmayo Largueta' and 'Wawona' (5% of germination), whereas in the case of 'Del Cid' it did not occur until week 6. The higher increase of germination corresponded to weeks 6 and 7 in 'Desmayo Largueta', weeks 6, 7 and 8 in 'Del Cid', and weeks 9, 10 and 11 in 'Wawona'. For later weeks, the germination percentages showed a lower increase. At week 15, the percentages of germination were 87% for 'Desmayo Largueta', 93% for 'Del Cid' and 98% for 'Wawona'. So, these cultivars showed a high percentage of germination.

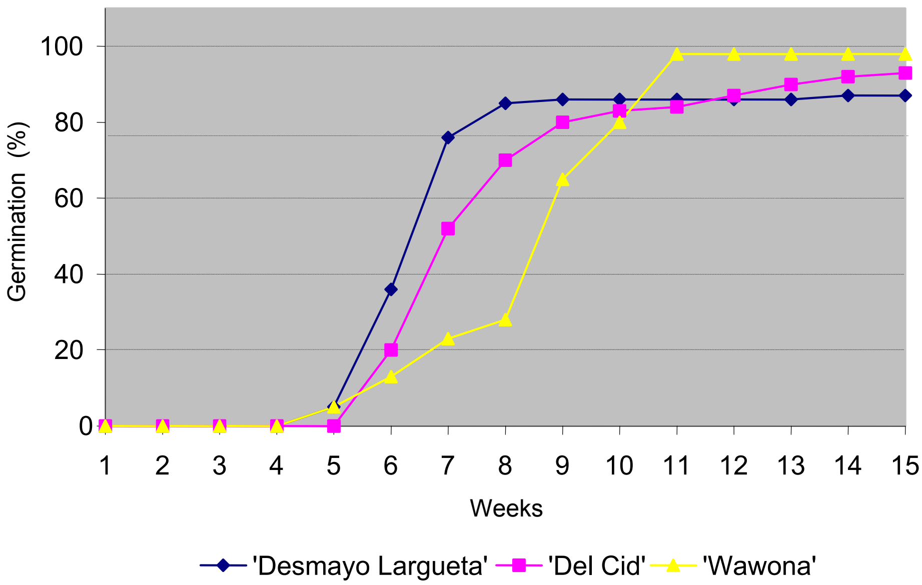

Fig.1. Accumulated percentages of seed germination (with endocarp) of 3 almond cultivars stratified for 15 weeks at 7 ºC.

Figure 2 shows the accumulated percentages of seed germination for the three studied cultivars at each week for the second treatment. Germination started after the fourth week when seeds were treated at 22ºC. At week 5, the percentage of seed germination showed an important increase. Percentages of germination reached 59% in 'Desmayo Largueta', 33% in 'Del Cid' and 17% in 'Wawona'. These percentages were higher than those obtained at week 5 for the first treatment. After removing the endocarp and the tegument, at week 11, the percentage of germination increased gradually between weeks 12 and 13, one or two weeks after the endocarp and tegument removal. The final percentage of germination in this second assay was also high for the cultivars 'Desmayo Largueta' (87%) and 'Del Cid' (98%). The low percentage of seed germination (44%) observed in 'Wawona' was due to a fungus attack, despite the fungicide treatment.

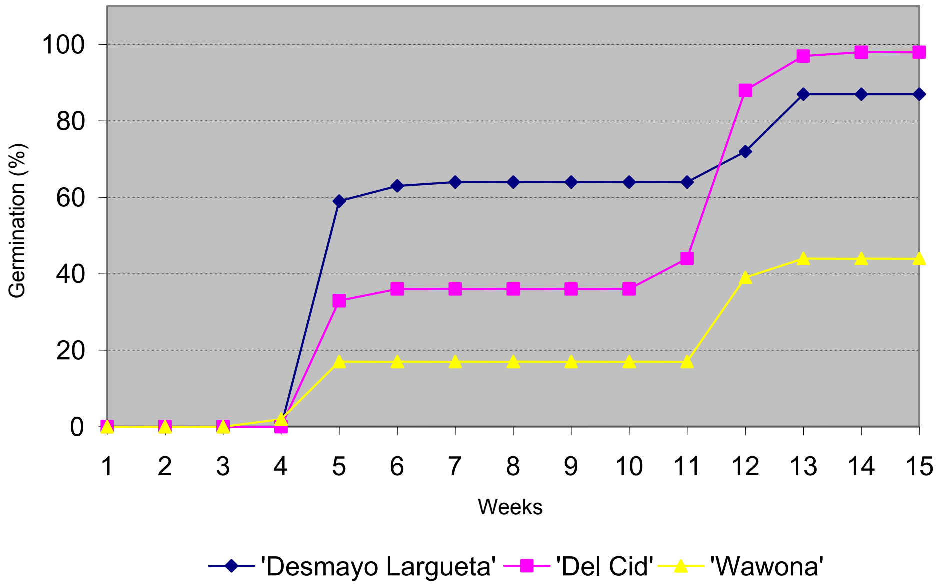

Fig. 2. Accumulated percentages of seed germination of 3 almond cultivars stratified for 4 weeks at 7ºC, followed by 11 weeks at 22ºC. In week 10, the endocarp and the tegument were removed from the nuts.

Despite the great variability observed in both assays, a relationship between chilling requirements of genotypes for flowering and stratification requirements of seeds for germination was observed. Seeds of 'Desmayo Largueta' germinated earlier, followed by 'Del Cid', and finally 'Wawona'. In the second assay, 4 weeks of stratification were not enough to break the dormancy of all seeds, mainly in the case of the late cultivar 'Wawona'. After this stratification period, those seeds that had fulfilled their stratification requirements germinated at 22ºC. Finally, seed dormancy was overcome after removing the endocarp and the tegument and the percentage germination reached the values observed in the first assay, a traditional stratification treatment.

Regarding the stratification temperature, the model of chill accumulation described by Hutchins (1950) established 7ºC as the highest temperature for breaking dormancy. However, Zehni (1963) determined 10ºC as the optimum temperature to promote seed germination when studying a great range of temperatures (from 2ºC to 10ºC) for stratification of almond seeds. Three weeks of stratification at 10ºC gave the same percentage of germination as stratification for 5 weeks at 2ºC. Seeley *et al*. (1998) observed in peach that cyclic temperatures combining 2ºC and 14ºC in cycles of 12/12, 14/10, 16/8 and 18/6 hours, gave higher percentages of germination and better seedling growth than constant temperatures of 2ºC, 6ºC or 14ºC. This could be due to the activation of growth promoters at 14ºC during the stratification process. On the other hand, Frisby and Seeley (1993) observed that peach seeds stratified at 2ºC germinated earlier than those stratified at 8ºC or 10ºC, although with abnormal growth. These results support the complementary effect of high temperatures on the germination of seeds previously stratified at cold temperatures.

Regarding the effect of the removal of endocarp and tegument, in agreement with our results, Zigas and Coombe (1977) demonstrated the inhibitory effect of tegument in the germination of peach seeds. In this way, Seeley *et al.* (1998) and Martínez-Gómez and Dicenta (2001) characterised the seed dormancy in peach as two independent mechanisms: tegument dormancy (external), with a hormonal nature and manifested in the inhibition of germination, and embryo dormancy (internal) with a genetic nature and expressed mainly in the later plant growth.

Stratification has been the method traditionally used to break external seed dormancy in *Prunus* species (Kamininski and Rom, 1973; Zigas and Coombe, 1977; Mehanna *et al.*, 1985; Seeley and Damavandy, 1985; Frisby and Seeley, 1993; Seeley *et al.*, 1998). In order to accelerate this process, the application of hormones (Chao and Walker, 1966; Rouskas *et al.*, 1980) and the removal of the tegument (Zigas and Commbe, 1977; Du Toit *et al.*, 1979; Rouskas *et al.*, 1980) have been used. Chao and Walker (1966) also indicated a mechanical effect of the tegument, with higher percentages of apricot and peach seed germination when it was removed. In almond seeds, González-Cepeda (1975) correlated the stratification requirements for germination with the concentrations of several hormones, such as abscisic acid (ABA), indicating an optimum balance between inhibitors and promoters.

Studies of the simultaneous effects of tegument and temperatures in the stratification requirements of seeds for germination are very scarce. Zigas and Coombe (1977) confirmed the inhibitory effect of the tegument on seed germination and the subsequent seedling growth. This inhibitory effect could be cancelled after stratification at 3ºC for 10 weeks. Removal of the tegument before stratification did not have any effect on seed germination. However, a greater growth of seedlings was observed.

In conclusion, our results show the different effects of stratification treatments on seed germination. A correlation between the stratification requirements for seed germination and the flowering date of the cultivar was observed. Heat treatment did not induce germination in those seeds that had not fulfilled their chilling requirements. However, removal of the endocarp and the tegument in seeds that had not fulfilled their chilling requirements produced the same effect as stratification.

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