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Role of the Endocarp in 'Manzanillo' Olive Seed Germination

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Abstract. **The endocarp of 'Manzanillo' olive (*Olea europaea* L.) seeds was subjected to several treatments in order to determine its effect on germination of the olive seed. The endocarp inhibited germination in stratified as well as unstratified olive seeds. Removing the endocarp resulted in high percentages of germination, but only when it was completely removed or when the radicle end was removed. The endocarp did not inhibit germination by preventing imbibition, since water uptake occurred in the seed through the untreated endocarp and through the clipped cotyledon end. The endocarp also did not contain water soluble inhibitors that prevent germination. Rather, the endocarp seemed to inhibit germination through mechanical resistance. High percentages of germination can occur only when the structure of the endocarp is altered, reducing its resistance to embryo expansion.**

Both wild and cultivated olive seeds, intact with endocarp, show low germination percentages and delayed germination under favorable conditions (1, 6, 8). Germination percentage of seed with endocarp is not increased by stratification, although stratification at 15°C for 30 days has been shown to break dormancy of the intact seed (embryo with inner seed coats) (4, 5, 9). In addition to the dormancy imposed by the inner seed coat (9), poor germination of olive seeds also can be attributed to the effect of the endocarp, the layer of stone cells that encloses the embryo and inner seed coat (7). The endocarp could present a physical barrier to water uptake, oxygen diffusion, and mechanical resistance to embryo expansion, or contain inhibitors of germination. The objective of this study was to investigate the role of the endocarp in olive seed germination.

Materials and Methods

Seed material. Fruit were collected from 'Manzanillo' olive trees in the Davis Pomology Experimental Orchard in 1981. The exocarp was removed with a seed cleaner. Empty seeds were culled by immersing the seeds in a 30% (w/v) solution of NaCl. The seeds that floated were discarded.

Stratification. Olive seeds were mixed with moist vermiculite and placed in polyethylene bags for stratification. Half of the seeds in each treatment had their endocarps removed prior to stratification. Five replicates of 25 seeds each were stratified at 7°, 15°, and 25°C for 1, 2, 3, and 4 months in a growth chamber. Five samples, each containing 20 seeds, were stratified for 30 days at 15° in polyethylene bags containing vermiculite. After stratification, the endocarps were broken individually in a hand vise, and the seeds were germinated in the polyethylene bags at 25°. The control treatment consisted of samples which had been stratified but which did not have the endocarp broken. Final germination was determined when the seeds reached a stable germination percentage.

Scarification. Water uptake was determined gravimetrically. Five replications of 10 seeds each were soaked in 3% NaOH for 0, 12, 48, and 90 hr. Seeds were air-dried until original weight was reached, then immersed in tap water for 48 hr. Water uptake was measured hourly for 12 hr during the experiment and then every 6 hr for the duration of the experiment, and was expressed as the percentage of increase in seed weight over the original weight. All seeds then were stratified at 15° and germinated as described below.

Tests for seed viability were performed on samples from NaOH scarification treatments. Embryos with the inner seed coat intact were removed from the endocarp and were surface-sterilized (9). Four replicates of 15 embryos with intact seed coat were placed in plastic petri dishes on Whatman No. 1 filter paper moistened with 75% Thiram fungicide in a 2% aqueous slurry. The embryo was considered viable if the radicle had elongated 2 mm at the time of recording.

Embryo expansion was determined by clipping 2–3 mm off ends of endocarps, or until the embryo was visible, and soaking them in water for 48 hr. Five replicates of 15 seeds each were placed in each treatment. The percentage of germination was determined after the seeds were stratified at 15° for 30 days and germinated as described below.

Endocarp inhibitors. An aqueous extract from endocarp tis-

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sue was obtained by soaking the endocarp in water at 22°C [2 water : 1 endocarp (v/v)] for 48 hr. Excised embryos with an intact inner seed coat were stratified for 30 days at 15°. These embryos were soaked in the endocarp extract for 48 hr at 22° and then were placed in petri dishes for germination.

Germination. Seeds were germinated at 25°C under a 16-hr photoperiod in plastic petri dishes for various periods of time depending on the experiment. They were placed on 2 layers of moistened Whatman No. 1 filter paper. A seed was considered to have germinated when the radicle reached 1 cm.

Scanning electron microscopy. Intact seeds were treated by soaking in concentrated H_2SO_4 for 30 hr, in 3% NaOH for 48 hr, or in water to investigate the effect of these chemicals on the structure of the endocarp. The endocarps were removed after the chemical treatments and were air-dried. They were mounted on SEM stubs, and were coated with 40 nm gold. Samples were viewed under an ISI-DS130 (International Scientific Instruments) scanning electron microscope at 15 KV and 45° tilt.

Statistical analysis. One-way analysis of variance was conducted on germination percentages after arc sine transformation. Results are expressed in tables as germination percentages (untransformed). Significant differences among treatment means were calculated using least significant difference (LSD). The objective of the data analysis was to determine those treatments that differed significantly from controls.

Results

Germination occurred only in embryos (with inner seed coat) which had the endocarp removed prior to stratification and only when the stratification temperature was 15°C (Table 1).

Water uptake was greatest in seeds soaked in NaOH for 48 hr or longer and least in controls which had been soaked in water (Table 2). Seeds in all treatments showed an initial rapid increase in water uptake during the 1st 4–5 hr of imbibition, followed by a decrease in rate of water uptake during the next 5 hr. After 12 hr, there was a slight, but insignificant increase in the amount of water uptake for the different treatments. At 12, 48, and 90 hr there were significant differences in water uptake compared to the control (Table 2). The greatest increase in weight due to water uptake occurred in the 90-hr treatment with 3% NaOH. Germination percentage increased with an increase in water uptake for the 12-hr and 48-hr treatments (Table 2), with the greatest germination percentage occurring after a 48-hr treatment with NaOH (2).

Clipping the ends of seeds had a marked positive effect on germination, but only when the radicle end was cut (Table 3). Although germination of excised embryos was significantly greater 1 week after stratification compared to that of embryos which had the radicle end of the endocarp removed, this difference did not occur at 4 weeks (Table 3). Imbibition, monitored by measuring expansion of the embryo beyond the cut surface of the endocarp, occurred in all treatments in which clipping was done.

Breaking open the endocarp after stratification was extremely effective in producing high percentages of germination compared to seeds which had an intact endocarp. None of the controls germinated, compared to 90% of seeds which had the endocarp broken.

There was no inhibitory effect of the aqueous extract derived from the endocarp on germination of stratified embryos in the presence of the inner seed coat (Table 4). We did not use a nonaqueous solvent extraction of the endocarp because of the likelihood that aqueous inhibitors would be extracted during imbibition and affect germination of the embryo.

Both H_2SO_4 and NaOH modified the surface of the olive seeds as seen under SEM and under a dissecting microscope. H_2SO_4 etched most of the vascular bundles from the surface of the seeds and changed the appearance from the characteristic rough surface to a smooth surface. NaOH was less damaging to the surface and seemed to reduce the number of vascular bundles less than did the acid.

Discussion

The high germination percentage reached only at a stratification temperature of 15°C confirms previous reports by Lagarda (9) concerning the critical effect of stratification temperature on germination of olive seeds.

The weight increase by seeds soaked in water without pretreatment in NaOH indicated that the endocarp is permeable to water. Similar findings for 'Picholine' olive seeds were reported by Ruby (11) who found that maximum water uptake occurred between 10 and 17 hr of imbibition. Thus, poor germination cannot be attributed solely to a lack of imbibition due to impermeability of the endocarp.

NaOH may improve germination of olive seeds by making the endocarp more permeable to water, thereby allowing increased water imbibition. Previous reports indicated that olive seeds reach only 67% of their total imbibition capacity (3). The use of NaOH increased imbibition. Evidence from SEM indicated that NaOH could weaken the endocarp considerably, making it possible for increased water uptake to occur and for the embryo to split the stony endocarp during germination. Others also have shown that alkali can weaken a hard outer seed coat, thereby allowing the embryo to emerge (10).

The difference in germination percentage between cutting the radicle end and cutting the cotyledon end was not due to a lack of difference in water imbibition between the 2 treatments, since the embryo expanded in all 3 clipping treatments. Rather, it can be attributed directly to mechanical resistance of the endocarp

Table 1. Effect of stratification temperature and duration of stratification on germination of 'Manzanillo' olive seeds with and without endocarp.

	Germination %							
	Length of stratification (months)							
	1		2		3		4	
Stratification temperature (°C)	With endocarp	Without endocarp	With endocarp	Without endocarp	With endocarp	Without endocarp	With endocarp	Without endocarp
7	0	0	0	0	0	0	0	0
15	0	95	0	90	0	85	0	90
20	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0

Table 2. Effect of time of 3% NaOH treatment on water uptake and germination percentage of 'Manzanillo' olive seeds.

Time (hr)	Water uptake[z] (% increase in original weight)	Germination[z] (%)
0	14.6	0
12	18.3	13
48	21.5	58
90	24.5	0
LSD	3.1	24

[z]Data analyzed after arc sine transformation.

Table 3. Effect of clipping treatments on germination of stratified 'Manzanillo' olive seeds.

	Germination (%)	
	Time after stratification	
Clipping treatment	1 week	4 weeks
No clipping	0 c[z]	0 b
Cotyledon end cut off	0 c	0 b
Radicle end cut off	25 b	76 a
Both ends cut off	31 b	80 a
Excised embryo with inner coat	41 a	88 a

[z]Date analyzed after arc sine tranformation. Mean separation in columns by Duncan's multiple range test, 5% level.

Table 4. Effect of aqueous extract of endocarp during initial imbibition on germination percentage of stratified 'Manzanillo' olive seeds[z] without endocarp.

	Germination (%)				
	Time of measurement (days after stratification)				
Treatment	2	4	6	8	10
Control	44[y]	48	48	53	76
Extract	39	51	53	60	75

[z]1981 harvest, experiment conducted in 1982.
[y]No significant differences within columns.

which prevented radicle emergence. Clipping off the ends of the endocarp also showed that the lack of oxygen diffusion was not a significant factor in preventing germination, since oxygen could have entered easily when the cotyledon end was cut; yet, no germination occurred.

As it was applied, the aqueous extract derived from the endocarp was not effective in inhibiting germination once stratification had occurred. It may be possible that an inhibitor is present in the endocarp but acts prior to or during stratification, due to differential sensitivity of the embryo over time. Another possibility is that the inhibitors could have been diluted by the water in which the endocarps were soaked for the extraction. Further study of these hypotheses is necessary.

Our studies showed that internal requirements for germination were satisfied by stratification at 15° for 30 days, but the endocarp alone inhibited germination after stratification. That this was the case was demonstrated by the experiment in which the endocarp was removed after stratification; 90% germination was obtained from this treatment, compared to no germination in controls. These results, combined with those from the clipping treatments, strengthen our hypothesis that the endocarp effectively inhibits germination of olive seeds by mechanical resistance.

Since both scarification and stratification were necessary to achieve a high percentage of germination, we can conclude that 'Manzanillo' olive seeds have both a mechanical and physiological dormancy. Stratification removes the physiological dormancy which is reported to be caused by ABA in the inner seed coat (9). The endocarp acts as a mechanical barrier to embryo expansion. Only when the endocarp is destroyed in some way to allow radicle emergence can germination occur.

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