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Germination of 'Arabequina' Olive Seeds As Affected By Chemical Scarification, Hot Water Treatment And Endosperm Tissue

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ABSTRACT

The effect of scarifying olive *Olea europaea* L. cv. Arabequina endocarp containing seeds (stony seeds) with H_2SO_4 and NaOH at two concentrations (0.1N and 1N) and two soaking periods (24 hrs and 48 hrs) as well as water for 24 and 48 hrs and hot water 50°C and 100°C for 24 and 84 hrs on their germination was investigated. To study the effect of the endosperm on seed germination, *in vitro* embryo culture of the same cultivar was performed. The germination potential of isolated embryos was compared to that of complete seeds and to sections of seeds including 2/3, 1/2, and 1/3 seed sections. The highest significantly germination percentage (60%) was obtained when the stone seeds were scarified with 0.1 N H_2SO_4 for 24 hrs after 115 days of planting. Treating the stony seeds with either H_2SO_4 for 24 hrs or water at 50° C and tap water for 24 and 48 hrs raised germination percentage over other treatments. Soaking the stony seeds with H_2SO_4 for 48 hrs improved germination percentage but with lower effect than H_2SO_4 for 24 hrs. The excised embryos and the 1/2 seed portion started to germinate after one week of culturing. The highest embryo germination percentage (70%) was recorded after one month of planting. Both 1/2 and 1/3 seed's sections exhibited higher germination percentage than the germination of 2/3 seed section or whole seed. The results of this work indicated a pronounce effect of the endocarp and endosperm on olive seed germination.

KEYWORDS: Seed dormancy, *Olea europaea*, Scarification, *In vitro*.

INTRODUCTION

Olive is an important economic crop for many Mediterranean countries. Olive trees were mainly propagated by grafting desired cultivars onto seedling rootstocks. At present, most olive cultivars are propagated by semi-hard wood cuttings elsewhere in the world (Caballero, 1994). The reasons for that shift are the difficulties in obtaining seedling rootstocks. For instance, olive seed germination is erratic, very slow and proceed for 2-3 years (Sotomayor-Leon and Caballero, 1990; Zuccherelli and Zuccherelli, 2002) and germination percentage might not exceed 10% in many cultivars (Acebedo et al., 1997).

The major barrier for olive seed germination is the stony endocarp in addition to other causes of dormancy including seed coat, endosperm and embryo itself (Lagarda et al., 1983a, Lagarda and Martin, 1983; Prista et al., 1999 and Lagarda et al., 1983b). Seed germination of 'Manzinallo' olive was improved by using stonless seeds (Crisosto and Sutter, 1985). However, low germination percentage was obtained with other cultivars using stonless seeds (Acebedo et al., 1997). It was reported that 28% of olive seed dormancy is imposed by the endocarp and 56% by the endosperm (Sotomayor-Leon and Caballero, 1994). For commercial olive seed germination, breaking olive endocarps described by

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Sotomayor-Leon and Caballero (1990) does not always work well. Chemical scarification has been widely used to overcome physical seed dormancy (Hartmann and Kester, 2002). Germination percentage of three olive cultivars was improved after the stony seeds were scarified with 0.1 N NaOH and H_2SO_4 (Bandino et al., 1999).

Endosperm effect on seed germination is mainly overcome by seed cold stratification for different durations (Sotomayor-Leon and Caballero, 1999; Lagarda and Martin, 1983; Acebedo et al., 1997; Brhadda et al., 1999). Chemical agents such as norflurazon and continuous washing in running water have also been used to overcome olive seed dormancy (Sotomayor- Leon and Altisent, 1994). The effect of endosperm on embryo dormancy was demonstrated by *in vitro* germination of isolated olive embryo where up to 100% germination was achieved within 10-14 days (Acebedo et al., 1997; Rugini, 1986; 1990; Lagarda et al., 1983b).

The aim of this work was to determine the effect of different treatments on the germination of 'Arabequina' olive seeds as well as to investigate the effect of endosperm on embryo germination of the same cultivar.

MATERIALS AND METHODS

Fruits from 'Arabequina' olive cultivar from non irrigated olive orchard at the Faculty of Agriculture Campus, Tulkarem, An-Najah National University were harvested in October, 2003 when the color was changed from yellow green to violet. Fruit flesh was separated by soaking them in 4% NaOH. The stony seeds were cleaned with sand and water, and were then soaked in running water for 12 hrs to remove residues. The stony seeds were mixed with moist vermiculite inside a perforated polyethylene containers and placed in a cold chamber at 4-6 C° for one month before scarification or *in vitro* culture. The stratified stony seeds were exposed to different scarification treatments. The treatments were; NaOH or H_2SO_4 at 0.1 or 1 N concentration for two soaking periods (24 and 48 hrs). A second group of stony seeds were soaked in hot water at 50° C or 100°C. A third group was soaked in water (room temperature) for 24 hrs, while a fourth group was soaked for 48 hrs as a control treatment. Therefore, a total of twelve treatments was used (Table 1). Fifty seeds were used in each treatment with five replicates for each. The stony seeds in the 12 treatments were left to germinate in plates filled with a mixture of 1:1 peat to vermiculite and placed in a greenhouse at 20 to 25°C. Germination was determined by the emergence of the hypocotyle. Statistical analysis was done using SAS software package (SAS Inst. 1990), for mean differences the analysis of variance was followed by LSD test at 5% probability level.

For *in vitro* culture the seeds endocarp were broken as described by Sotomayor-Leon and Caballero (1990), seeds were surface sterilized with 20% Clorox (a.i. 5.25% sodium hypochlorite), and rinsed in sterile distilled water for 24 hrs. The seeds were then divided into five groups; complete seed, 2/3 seed with embryo, 1/2 seed with embryo, 1/3 seed with embryo and single embryo (Table 2). The embryo were isolated as described by Acebedo et al. (1997). Fifty portions of each group were then planted aseptically onto 1/2 MS medium (Murashige and Skoog, 1962) in sterile 18x25 mm test tubes. The cultures were then incubated in a growth chamber at 23± 2C° for 16 hrs light period with a light intensity of 40μ E m^{-2} S^{-1}. Germination of *in vitro* culture was determined by the emergence of the radical and opening of the cotyledons. For statistical analysis, Chi-Square test was performed.

RESULTS AND DISCUSSIONS

1. Scarification experiment:

The statistical analysis of the germination percentage indicated significant differences among different treatments (Table 1). Stony seeds soaked in 0.1 N H_2SO_4 for 24 hrs gave significantly the highest germination percentage (60%) after 115 days of planting. The stony seeds that were scarified with 0.1 N H_2SO_4 (48 hr), 1N H_2SO_4 (24 hrs) and 50°C water (24 hrs) started to

germinate after 75 days of planting with only 8, 6, and 2% of germination percentages, respectively. After 85 days of planting, scarification treatments of 0.1 N H_2SO_4 (24 hrs), 1N H_2SO_4 were on the same level of significance (14% and 12%), other treatments gave no or low germination. Similar trend was exhibited at the later counting dates, with the highest germination percentage obtained from seeds scarified with 0.1 N H_2SO_4 (24 hrs). Exposure of the stony seeds to longer duration of H_2SO_4 or and higher concentrations reduced seed germination (Table 1). For instance, seeds scarified with 1N H_2SO_4, for 24 and 48 hrs gave significantly lower seed germination (32 and 8%), respectively at 125 days. Seeds that were scarified with NaOH at both concentrations and soaking duration exhibited significantly the lowest germination potential at all counting dates. Seeds on the control treatments germinated after 95 days of planting, it continued to germinate reaching a germination percentage of 30% at the last counting date. No germination was observed with stony seeds treated with hot water 100° C, however, seeds treated with water at 50° C gave a germination percentage 28% at 115 days which was at the same level of significance with 0.1 N H_2SO_4 (48 hrs) and significantly lower than 0.1N H_2SO_4 (24 hrs).

In this experiment, H_2SO_4 was effective at 0.1 N concentration and 24 hrs soaking in reducing the seed dormancy. Olive seeds of most cultivars usually had low germination (Sotomayor-Leon and Caballero, 1990) and the removal of the stony endocarp improved seed germination (Rugini, 1986). However, the complete removal of the endocarp is not easy, therefore, partial removal of the endocarp by chemical or mechanical treatments could be effective. The corrosive effect of H_2SO_4 was most evident at the surfaces of the seeds. Sulfuric acid is thought to disrupt the seed coat and expose the lumens of the macrosclereids cells, subsequently permitting water imbibition (Aliero, 2004). A 100% water seed imbibition of *Chordospartium stevensonii* was achieved after acid scarification, where only 5% imbibition occurred in unscarified seeds (Conner and Conner, 1988). On the other hand, higher levels and duration of acid exposure might cause damages to the embryo. In our experiment, high concentration of H_2SO_4 or NaOH as well as hot water reduced germination. Herron and Clemens (2001) observed a similar reduction in seed germination following acid scarification of *Melicytus ramiflorus* seeds, similar results were reported by Bannister and Bridgman (1991) with three species of *Pseudopanax*. In our results, the dip in boiling water (100°C) could have caused damages to the embryo or the treatment was not effective. Sudden dip of seeds in boiling water is usually effective against seed coat (Aliero, 2004).

2. In vitro culture:

The Chi-Square test analysis indicated a highly significant differences among treatments (Table 2). The highest germination percentage was obtained from isolated embryos (70%) after 4 weeks of culturing. Germination of 1/2 seed portions and the isolated embryos started one week after culturing with 10 and 40% germination percentages (Table 2). Other portions (1/3 and 2/3) started to germinate after three weeks of culturing with 10 % germination percentage each. At the last counting date, 1/2, 1/3 and single embryo portions resulted in a higher germination percentages compared to 2/3 or complete seed. The complete seed started to germinate after 6 weeks of culturing, achieving 10% at week 6.

These results are in agreement with the previous work of Acebedo et al. (1997) who obtained 70-80% embryo germination level with 10 olive cultivars and Lagarda et al. (1983a) who obtained 100% embryo germination of 'Manzanillo' olive cultivar. The inhibitory effect of seed germination might be found in the seed coat and the endosperm, the dormancy of 'Arabequina' stonless olive seeds was completely broken by 50µM norflurazon and 30 days of continuous washing in running water (Sotomayor- Leon and Altisent, 1994).

The result of this work indicated the inhibitory effect

of the endosperm which matched with the reports of Sotomayor- Leon and Altisent (1994); Maalej et al. (2002) who found that using 1/2 olive kernel increases the germination of three olive cultivars. In another study, a higher olive embryo germination (69%) was obtained compared to only 6% germination of the stonless seeds of Moroccan Picholine variety (Brhadda et al., 2000).

Dormancy might also occur in the isolated embryo (Voyitzis and Prista, 1994; Voyitzis, 1995; Brhadda et al., 2000), however, this might be cultivar dependent, embryo dormancy was not found in 'Manzanillo' olive seeds (Lagarda et al., 1983 b). In our study, embryo germinated after one week of culture, the stony seeds were exposed to one month chilling which apparently was enough for embryo to germinate, therefore it could be assumed that the seeds need more chilling period.

The result of this study showed that both endocarp and endosperm act as barriers of olive seed germination (Table1). This finding matches the previous work of Crisosto and Sutter (1985); Acebedo et al. (1997); Lagarda et al. (1983b); and Voyitzis (1995).

Olive stony seeds scarified with H_2SO_4 at 0.1 N for 24 hrs and the culturing of the isolated embryo could be a useful protocol for enhancing olive seed germination. The difference in the germination percentage obtained in our results was compared with other reports which could be due to stress conditions since the seeds were collected from non- irrigated trees (Acebedo et al., 1997). Environmental conditions during seed development have shown to be very important in determining the degree of hardness of seed coats in several species (Gutterman, 2000). Differences in seed germination behavior were observed in *Opuntia tomentosa* from year to year, seeds collected in the year 2000, needed only 5 minutes acid scarification to give a high germination percentage, while seeds of the same age collected in 1998 required 90 minutes in sulfuric acid to reach similar germination percentage (Olvera-Carrillo et al., 2003).

In spite of these results, more investigation is needed in the future to verify more treatments. Scanning electron micrographs will be useful to clarify the acid effect on the seeds. In addition, studies to extract inhibitors from seed tissues could be also investigated.

Table (1): Germination percentage of 'Arabequina' stratified olive stony seeds exposed to different scarification treatments.

	Days after planting					
Treatment	75	85	95	105	115	125
water (24h)	0 b*	8 bc	12 b	28 b	28 bc	30 bc
water (48h)	0 b	0 c	20 ab	30 b	35 b	35 bc
0.1 N NaOH (24h)	0 b	0 c	2 c	10 c	16 cd	18 cd
0.1 N NaOH (48h)	0 b	0 c	0 c	2 c	2 de	2c
1 N NaOH (24h)	0 b	0 c	0 c	0 c	6 de	6 de
1N NaOH (48)	0 b	0 c	0 c	10 c	16 de	10 dc
0.1 N H_2SO_4 (24h)	0 b	14 a	26 a	52 a	60 a	60 a
0.1 N H_2SO_4 (48h)	8 a	10 b	20 ab	36 b	38 b	40 b
1N H_2SO_4 (24h)	6 a b	12 ab	14 b	30 b	32 b	32 bc
1 N H_2SO_4 (48h)	0 b	0 c	0 c	4 c	4 de	8 dc
50 °C water (24h)	2 b	2 c	16 b	24 b	28 bc	28 bc
100°C water (24)	0 b	0 c	0 c	0 c	0 e	0 e

* Numbers followed by the same letter(s) within columns are not significantly different at 5% level according to LSD.

Table 2: *In vitro* germination of 'Arabequina' olive seeds of different portions .

Germination percentage (Days after planting)					
Treatment	Week2	Week3	Week4	Week5	Week6
Complete seed	0	0	0	0	10
2/3 seed with the embryo	0	10	20	30	30
1/2 seed with the embryo	10	20	45	45	45
1/3 seed with the embryo	0	10	30	50	50
Single embryo	40	50	60	70	70
chi-square value	30.74	23.42	19.62	22.64	16.53
P-value	0.000	0.000	0.001	0.000	0.002

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تأثير معاملات تخديش كيماوي مختلفة ونسيج الاندوسبرم على انبات بذور الزيتون/صنف(عرب كوين)

*حسان ابو قاعود**

ملخـــص

تمت دراسة تأثير معاملات تخديش كيماوية باستخدام حامض الكبريتيك والصودا الكاوية بتراكيز (0.1 ، 1معياري) وفترات نقع مختلفة (24، 48 ساعة) باستخدام مياه ساخنة على درجتي حرارة 50م و 100م وماء عادي (شاهد) على إنبات بذور صنف الزيتون عرب كوين ، كذلك تمت دراسة تأثير أنسجة الاندوسبرم على إنبات البذور وذلك بمقارنة إنبات أجنة البذور مع البذور الكاملة وأجزاء مختلفة من البذور (3/2 ، 2/1 ، 3/1) بحيث يحتوي كل جزء على الجنين. حصلت أعلى نسبة إنبات 60% بعد 115 يوماً من الزراعة باستخدام حامض الكبريتيك تركيز (0.1 معياري) لمدة 24 ساعة. أدى نقع البذور بحامض الكبريتيك لمدة 24 ساعة ومعاملة الماء عند درجة حرارة 50م والماء العادي (الشاهد) لمدة 24 و 48 ساعة إلى تحسين نسبة الإنبات.بدأت الأجنة المستخلصة من البذور بالإنبات بعد زراعتها بالأنابيب بعد أسبوع من الزراعة وكذلك جزء البذرة (2/1)، حيث وصلت أعلى نسبة إنبات للأجنة (70%) بعد شهر واحد من الزراعة.وكذلك أعطت الأجزاء 2/1 و3/1 نسبة إنبات أعلى من الأجزاء الأخرى. دلت نتائج الدراسة على التأثير الواضح للغلاف الخارجي (الاندوكارب) على إنبات البذور وكذلك نسيج الاندوسبرم المحيط بالجنين، وساهمت معاملة الحامض بتحسين نسبة الإنبات وكذلك الاجنة المستخلصة، وإزالة أجزاء من الاندوسبرم أدت إلى تحسين نسبة الإنبات في الصنف المذكور.

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