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Effects of tegument, endosperm, cold treatment and harvest date on germination of wild olive

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Abstract: Wild olive seeds (*Olea europaea* L. var. *sylvestris*), called oleaster do not germinate when placed under favourable conditions. In a series of experiments the effects of the harvest date, the endosperm, the tegument, and the cold treatment were evaluated on germination of seeds and embryos. The germination percentage of embryos and seeds harvested at different harvest dates increased during October month, these percentages decreased during November month, whereas no seeds and embryos harvested on the middle of December germinated. Embryo germinability was always higher than seed germinability, and this may be due to an inhibiting effect of the teguments and the endosperm on seed germination. Such dormancy, which gradually increased during maturation, could reside mainly in the endosperm and partly within the embryo. The cold treatment at 4°C for four to thirteen days increased seed and embryo germinability, whereas lengthening time at this temperature showed a negative effect on seed germination. The germination of seeds and embryos from six wild olive trees was also examined by recording the germination percentage and minimum imbibition time (T_{mi}).

Additional key words: oleaster, dormancy, mechanical scarification.

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Introduction

Olea europaea subsp. *europaea* L. comprises two varieties: cultivated and wild olive trees, respectively called *europaea* (cultivars) and *sylvestris* (oleaster) (Green and and Wickens 1989). Olive (*Olea europaea* L.) is the most widespread tree species in Mediterranean basin. In Tunisia, the olive is a commercially important fruit tree, about 60 million trees are distributed and spread onto 1.6 million ha, representing one third of arable lands of the country (DGPA/ONH 1996).

Wild olive trees, the progeny of an uncontrolled outcrossing are well adapted to difficult Tunisian climate. Several olive cultivars were grafted on wild olive trees which were interest genetic resources deserving to be conserved. The wild olive tree is valuable because provides shelter for different birds and wild plants in harsh environment.

Most perennial plants possess two modes of regeneration: sexual reproduction through seed and clonal reproduction through some form of vegetative propagation (Richards 1986). Clonal offspring are usually much larger than offspring produced through sexual reproduction (Starfinger and Stöcklin, 1996).

Olive trees are propagated by cuttings or grafting. Clonal propagation enables to multiply a single tree based on cuttings or suckers (Scaramuzzi and Roselli 1986) to obtain homogenous olive crop characterized by interesting features traits such as high yield, disease resistance, and oil composition. Consequently, orchards are more or less genetically homogenous for the main clone.

Clonal propagation does not allow genotypes combining new traits. Therefore, this method could break genetic progresses except for new mutations that are extremely rare. The variability estimation with vegetative propagated plants is often only partially transmitted. It derives from existing chimeric situation in the plant and some phenotypical characters cannot be transmitted due to their absence in sporogenic tissue (Morettini 1954). Moreover, clonal selection may lead to reduction of variability, whereas seed propagation permit to introduce new characters, to solve the problem of senescence of the variety (Nozeron and Chausset 1975) and to obtain, from free or controlled hybridization, the new genotypes adapted better to the environmental conditions, as in other fruit-bearing species, or plants characterized (Prataviera 1998).

However, as reported by several authors, olive seeds germinate with difficulty (Ruby 1916; Istanbouli 1974). Indeed, the mean time for germination ranged from 81 to 300 days of seeds and from 84 to 550 days of the cores (Ruby 1916; Loussert and Brousse 1978). Several olive varieties were grafted on wild olive trees which were more resistant to environmental conditions and diseases. To date, little is known about wild olive propagation by seed and the production of new varieties with interested characteristics. Oleaster seeds do not germinate promptly when placed under favourable conditions. This germination difficulty is a problem in raising plants for breeding or experimental purposes.

In Tunisia the wild olive trees dispread both in natural and agro-ecosystems (Hannachi et al. 2008, 2010). In this study we chosen a natural ecosystem represented by the Ichkeul Park. The wild olive trees, in this locality, were in natural association with pistachio (*Pistacia lentiscus*) and they are isolated from all cultural practices. The aim of the present work was to determine the influence of the harvest date, the tegument, the endosperm and cold treatment on wild olive seed germination.

Material and methods

Seed preparation

Olives were collected from wild olive trees grown in Ichkeul Park in North Tunisian. The olives were harvested from the same tree at different date, from October to December, every two weeks, for testing the effect of harvest date on germination. The collected olives were stoned by hand separation: the olives were opened by cutting the pulp with a knife and the stones were simply scooped out. This technique applied to extract the seeds from the stones is fundamental to keep the seed intact to avoid damage to the embryos. The seeds were washed and dried with the ambient air. After the seeds were disinfected with 1% sodium hypochlorite, and then rinsed several times with sterile distilled water.

Seeds without tegument and with endosperm were prepared. Precisely, under sterile conditions, the tegument was carefully removed using a sharp blade taking intact the endosperm. At the end of this operation, these seeds without tegument were immediately placed in Petri-dishes.

1. Petri-dishes STE: The seeds were placed in Petri-dishes on two layers of filter paper (Whatman) moistened to saturation with sterile distilled water, so that germination was not limited by water. Additional sterile distilled water was added when needed, care was taken to not inundate the seeds. Petri-dishes were sealed with parafilm to minimise water loss. 50 seeds were used per Petri-dish.

2. Petri-dishes SE: the seeds were removed from their teguments, leaving intact endosperm, they were then cultured on Petri-dishes according the protocol described above. 50 SE (seeds without teguments) were used per Petri-dish.

Petri-dishes were then placed in controlled environment chamber under a photoperiod of 16 h of light/8 h of darkness at 18 ± 1°C as determined previously by Istanbouli (1985).

Embryos preparation

From the seeds prepared according the protocol described above, embryos were excised under sterile conditions. They were removed from their involucres (tegument and endosperm) using a sharp blade without wounding them. At the end of this operation, the embryos were immediately sown in Petri-dishes containing two filter papers soaked with sterile distilled water (Istanbouli 1985). The cultures were maintained in the same environmental conditions described above for the seeds. All these experiment tests were conducted on triplicate.

The effects of tegument and endosperm on germination were studied on two lots of 50 seeds (STE seeds with teguments), 50 seeds (SE seeds without teguments) and 50 embryos (E).

The influence of the olive cold treatment was studied on: seeds (STE) and embryos (E) which were prepared from the olives collected at harvest date (third week of October). The cold treatment ranged from four days to 14 weeks. Every week, seeds (STE) and embryos (E) were put to germinate.

Effect of genotype on oleaster seeds germination

Six oleaster trees were used to test the genotype effect on germination. For each tree two Petri-dishes were used in triplicate: seeds (50 STE) and embryos (50 E).

Germination was defined as the first emergence of the radicle. Germination count was made every 3 days for a period of 90 days. The germination process was monitored every day and the seeds infected by fungi or bacteria were removed.

Statistical analysis

The parameters taken in consideration were the germination percentage and the minimum imbibition time (T_{mi}):

– The germination percentage refers to the percentage of seeds able to germinate under experimental conditions and indicates the success of germination under a particular treatment:

$$G\% = 100x\frac{SG}{IS}$$

where

SG – number of germinated seeds

IS – number of seeds initiated in each test

The percentage of cumulative seed germination (G%) for each test was calculated at the end of the experiment.

– Minimum imbibition time (T_{mi}) which is the minimum time required for the seeds to start germinating since they have absorbed the necessary amount of water.

Mean Differences on seed and embryos germination of six wild olives were compared using ANOVA analysis. All tests were carried out in triplicate and the results were presented as means ± Standard Deviation (SD) using analysis of variance. Differences were considered statistically significant at $p < 0.05$ by Duncan's multiple-range test. Statistical analysis was performed using the software Statistica (Sta Soft Inc., Johannesburg, ZA).

Results

Effects of tegument and endosperm on germination

The seeds and the embryos placed under the same experimental conditions did not germinate at the same time (Fig. 1). Embryos excised from oleaster seeds germinated rapidly after 4 days from the experiment started. Specially, the simultaneous elimination of the tegument and the endosperm showed a remarkably decreased of T_{mi} until to 4 days, whereas the only elimination of the tegument (SE) reduced the T_{mi} by comparison with seeds (STE) from 18 to 8 days. Final germination percentage was different among seeds (STE), seeds without teguments (SE) and embryos (E): 20, 43 and 84%, respectively (Fig. 1).

Influence of the harvested date on germination

The germination percentage of seeds and embryos increased during October month, from 15% in seeds harvested on the first week to 48% in seeds harvested at the end of October, this percentage decreased to 10% at the end of November whereas no seeds germinated on the second week of December (Fig. 2). The germinability of embryos excised from seeds harvested at different dates increased from 20% on the first date to 80% in seeds harvested at the end of October, after this date the germination percentage decreased up to 20% at the end of November and no embryos harvested on the last dates germinated. At different harvest dates, the embryos showed higher percentage germination than seeds.

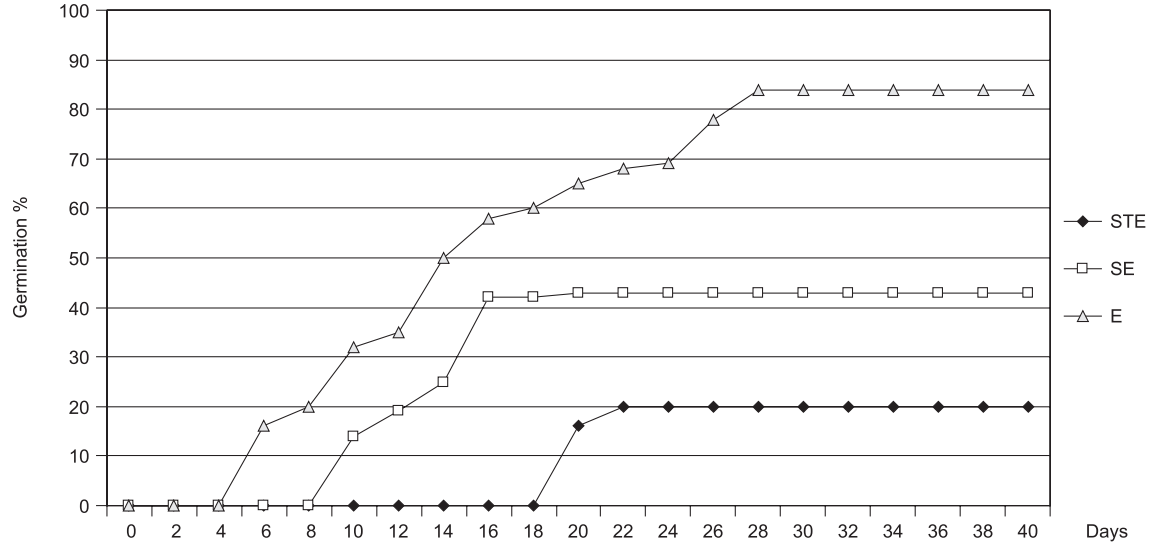

Fig. 1. Effect of tegument and endosperm on oleaster seeds germination at 18°C
STE: seed with tegument and endosperm; SE: seed without tegument; E: naked embryos.

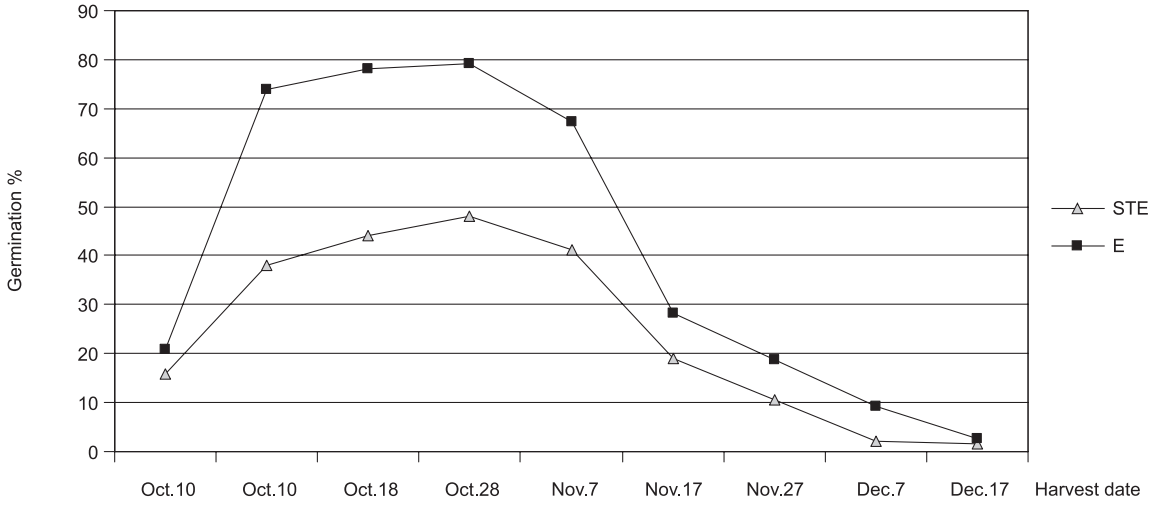

Fig. 2. Effect of harvest date on oleaster seeds germination at 18°C
STE: seed with tegument and endosperm; E: naked embryos.

Effects of cold treatment on germination

The conservation of the oleaster olives at 4°C during two weeks (from 4 to13 days) seemed to be the most favourable condition for the germination of seeds and embryos, 47% and 94%, respectively (Fig. 3). This treatment, compared at not treated seeds (Fig. 1), increased the germination percentage in seeds from 20 to 47% and in embryos from 84 to 94%. After two weeks, the percentage of germination was inversely correlated with cold treatment. The curves of seeds and embryos shows three phases in figure 3:

– Phase 1. the cold treatment ranged from 4 to 13 days improved germination percentage of both seeds and embryos. The highest germination percentage of seeds and embryos were 47% and 94%, respectively.
– Phase 2. the cold treatment ranged from 13 to 27 days, decreased moderately the germination percentage both in embryos and in seeds.
– Phase 3. the cold treatment ranged from 30 to 80 days, decreased remarkably the germination percentage till only 1% on seeds and 16% on embryos at the end of experimental test. This could be due to the fact that seeds have three inhibitors simultaneous: the tegument, the endosperm and cold treatment, whereas embryos have only the cold treatment.

Comparison of oleaster seeds germination

The germination percentage and minimum imbibition time (T_{mi}) both of the seeds (STE) and the embryos (E) from all six oleaster trees have shown in Ta-

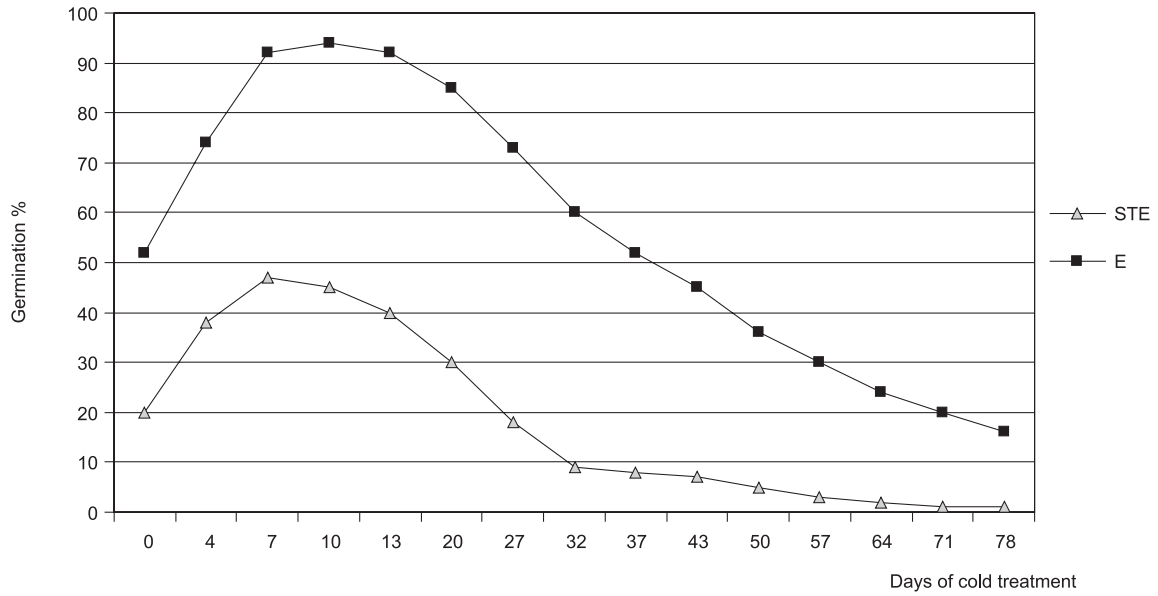

Fig. 3. Effect of cold treatment of olives at 4°C before oleaster seeds germination at 18°C
STE: seed with tegument and endosperm; E: naked embryos.

Table 1. Germination percentage (G %) and minimum imbibition time (T_{mi}) of seeds (STE) and naked embryos (E) from six oleaster trees from Ichkeul park, coded from O1 to O6.

Tree number	Germination percentage (G %)		T_{mi} (days)	
	STE	E	T_{mi} STE	T_{mi} E
O1	14.86 ± 2.45^{b}	47.02 ± 2.17^{d}	39 ± 4^{d}	12 ± 3^{c}
O2	8.45 ± 3.78^{a}	28.75 ± 3.17^{b}	21 ± 3^{bc}	10 ± 5^{bc}
O3	12.48 ± 3.00^{b}	37.00 ± 4.19^{c}	23 ± 4^{c}	9 ± 2^{bc}
O4	29.89 ± 4.80^{d}	21.00 ± 4.87^{a}	14 ± 3^{a}	6 ± 2^{a}
O5	15.28 ± 2.98^{b}	50.00 ± 5.67^{d}	18 ± 3^{b}	6 ± 3^{a}
O6	22.67 ± 2.56^{c}	83.00 ± 4.67^{e}	12 ± 2^{a}	8 ± 2^{ab}

Each value in the table is represented as mean ± Standard Deviation (SD).
Superscript letters with different letters in the same column, respectively, indicate significant difference ($P < 0.05$) analyzed by Duncan's multiple range test.

ble 1. Germination percentage ranged from 8.45 (oleaster O2) to 29.89% (oleaster O4) for seeds and from 28.75 (oleaster O2) to 83% (oleaster O6) for embryos. The embryos showed the highest germination percentage compared to the seeds. The minimum imbibition time T_{mi} ranged from 12 to 39 days for seeds and from 6 to 12 days for embryos. Statistically, the ANOVA showed that the difference of germination percentage and the minimum imbibition time between oleaster trees were significant ($P < 0.05$)

Discussion

The removal of tegument and endosperm improved the germination percentage of wild olive. In fact when isolated from the seed, the embryos are able to germinate very rapidly. Further, embryo germinability was always higher than seed germinability, and this may be due to an inhibitor effect of tegument and endosperm. Therefore, seed scarification improves seed germination as reported by Rostami and Shasaver (2009). The seed-covering layers can impose a physical constraint (coat dormancy) to radicle protrusion, which must be overcome by the growth potential of the embryo (Bewley 1997a; Koornneef et al. 2002; Leubner-Metzger 2003; Kucera et al. 2005).

The tegument effect, evidenced on *Olea* seeds (Scaramuzzi 1958; Istanbouli 1974) and on other species like *Citrus* (Demni and Bouzid 1979), can be explained by the mechanical role, obstacle to water and oxygen circulation, and by the presence of hormonal substances having an inhibitor role on germination. The contribution of the endosperm to the extent of coat dormancy has to be considered (Hilhorst 1995; Bewley 1997a; Leubner-Metzger 2003; Corbineau and Côme 1980). In olive seeds, the incapacity to germinate due to endosperm was evidenced by Brhadda et al. (2000) in Moroccan Picholine cultivar. The endosperm weakening, promoted by gibberellins (GA) and partially abscisic acid (ABA), is part of germination process of nondormant seeds and is not part of a dormancy release process (Baskin and Baskin 2004).

This study has shown the importance of the harvest date on wild olives seed germination. As already evidenced on *Olea* seeds by other reports, germination capacity appeared linked to the stage of seed maturity, therefore the maturation state could be influence the germinate process. In fact, the germination increased during maturation from the first week of October till the end of October, after this date germination percentage decreased till to reach at the end of November the same value of first week of October, further no seeds harvested on the middle of December germinated. Similarly, the germinability of embryos isolated from seeds harvested at different stages of maturity increased from first date of harvest till to the end of October, after this date the germination percentage decreased till to reach the lowest values of germinability on the last date of harvest. This phenomenon can be explained by embryo dormancy (Istanbouli 1974). The embryos physiological state development contributes to germination process (Khabou and Trigui 1995; Liòán et al. 1999; Kiani et al. 2006). Embryo germinabiliy was always seen to be higher than seed germinability, and this may be due to an endosperm-imposed dormancy that gradually increased during maturation. The effect of harvesting date has been reported on seeds germination of cultivated olive (2000). The inhibitory influence of the endosperm and embryo dormancy have directly related to the level of endogenous inhibitors (Istanbouli and Neville 1979; Porlingis and Voyiatzis 1986). Seeds pass through various phases in the course of their development – histodifferenciation (initial morphogenesis), maturation (seed expansion) and maturation drying (dessication) (Muntz 1982). Dormancy in plant is a process where physiological activities cease in a reversible manner. Seed dormancy represent one of the least understood phenomena in the field of seed biology (Hilhorst 1995; Bewley 1997b), innate and enforced dormancy have been found in several species such as cactus (Drezner and Lazarus 2008).

The plant hormones are considered extremely important for the regulation of seed dormancy and germination (Koornneef et al. 2002; Finkelstein 2004).

Germination process begins with the uptake of water for seed hydration allowing only pre-germinate metabolic activation, but insufficient to permit radicle protrusion through the seed coat (Nascimento and Aragao 2004), role due to the embryo expansion. In this study, the uptake of water by a seed was triplicate, with a rapid initial uptake (phase I, imbibition) followed by a plateau phase (phase II). A further increase in water uptake (phase III) occurred only when germination is completed, as the embryo axis elongated and broke through its covering structures (Bewley 1997a; Manz et al. 2005).

In this study, the cold treatment of wild olives for a limited period improved seed germination. In fact, the inhibition influence started to install gradually after 14 days and almost totally after three months. Similarly, the cold treatment increased embryos germination till 14 days, after the germination gradually decreased till to reach low values. These results confirm the effectiveness of the cold treatment on wild seed germination as reported on *Olea* by Wallali (1971), further a longer treatment did not produce any effect on the embryos germination (Istanbouli 1985).

Large difference in germination percentage and the minimum imbibition time were observed within six oleaster seed provenances. Embryos isolated from seeds showed a higher germinability than seeds. The difficult to germinate could be due to the inhibitory influence of endosperm and in some varieties of embryo dormancy, another possibility could be explained by either the infertility of the seeds. Seed propagation in the olive is undertaken mainly for the purpose of producing seedling to be used as rootstocks for cultivated olive trees. Theses seedling were heterogeneous in term of vigour and root development.

This wide range of variation could be partly explained by differences between oleaster trees due essentially to genetic origin and and/or phenotypic origin (the local conditions of seed maturation). Further in most plant species, seed germination varies between and within populations and between and within individuals (Baskin and Baskin 1998; Bischoff et al. 2006).

The important feature of sexual propagation is genetic variability that increases the likelihood of at least some individual's survival in the hazards of natural selection (Fenner and Thompson 2005). The response pattern of seed germination is as a key characteristic in plant life history strategy (Mayer and Poljakoff-Mayber 1989), seed germination can be regulated not only through genotypic characteristics (Gutterman and Gendler 2005), but also by environmental conditions (Beardsell and Richards 1987). One of the most intriguing innovations during the evolution of vascular plants has been the ability to form seeds as propagation and dispersal units. The genetic, physiological and biochemical proprieties of seeds are most important for the survival of a wild plant species in an ecosystem.

Conclusion

The germination process is influenced by several factors. The tegument, endosperm, harvest date and cold treatment influenced wild olive germination. In the present study we noted that the optimum conditions for germination percentage higher than 80% were: i) using isolated embryos instead of seeds, ii) seeds harvest in third October week, iii) the treatment of seeds at 4°C for five to thirteen days. Wild olive propagation by seed is important to increase the genetic basis of wild olive trees and to create new progenies with interest characteristics.

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