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ORIGINAL PAPER

Desiccation and storage behavior of bay laurel (*Laurus nobilis* L.) seeds

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Abstract The effect of seed moisture content (m.c.) and seed storage conditions of bay laurel (*Laurus nobilis* L.) was investigated in relation to seed viability. In the first experiment, the effect of drying rate on seed moisture and seed germination was investigated. Fresh seeds, with their original moisture content displayed a germination percentage of 55.1%. When the seed moisture content was reduced by 2.0% in an oven, the germination percentage rose to 81.0%. When the seed moisture content was reduced even more by using the same method, the germination percentages decreased dramatically. Reducing the seed moisture content to 28.7 and 23.5% by drying the seeds in alternating room conditions resulted in an increase of seed germinability to 84.3 and 90.9%, respectively. The drying of the seeds for 45, 60 and 75 days reduced their seed germination to 66.8, 49.4 and 48.0%, respectively. Reducing seed moisture content below 15.0% resulted in practically nullifying seed germinability. The fact that bay laurel seeds cannot retain their germinability at lower moisture contents demonstrates that it is a species with recalcitrant seeds. In the second experiment, moist and dry storage conditions were tested under different temperatures and moisture contents. The storage experiment showed that the most effective way of conserving the bay laurel seeds is moist storage at 0 ± 1°C for 4 months without previous drying of the seeds.

Keywords Bay laurel · Desiccation · Germination · *Laurus nobilis* · Moist storage · Recalcitrant seed

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Introduction

Bay laurel (*Laurus nobilis* L.) is an evergreen aromatic shrub or small tree that is an indigenous species of the Mediterranean (Kavvadas 1959). It is a dioecious plant, whose fruit is a drupe with a single seed of 1–1.5 cm diameter, oval shaped and black when ripe. The plant has been known since ancient times and has been associated with several myths and considered a symbol of victory, glory and honor (Kavvadas 1959). The pharmaceutical properties of its leaves and fruit have also been known since Dioskorides' time (Skroubis 1990). Its utilization in cooking and perfumery plays an important role till date and is widely cultivated as an ornamental plant in gardens and parks (Arabatzis 1998). Moreover, it is used symbolically in religious celebrations (Baumann 1993). Despite its importance, there is not much previously published seed biology information on this species available.

Investigations of the bay laurel seed germination behavior have shown that the external fleshy pericarp causes dormancy (Takos 2001; Tilki 2004) while embryo dormancy is also referred (Sheryshov 1975; Mkervali 1977a, b; Vadochkoriya and Loladze 1986; Takos 2001; Tilki 2004). Seed dormancy is broken by soaking the seeds in water under room conditions, for approximately 10 days, in order to remove the pericarp and then stratifying the seeds at 4 ± 1°C for 30–45 days (Takos 2001; Tilki 2004).

The seeds of many plant species have an orthodox storage behavior (Dirr and Heuser 1987; Thanos and Georgiou 1988; Bonner 1990; Thanos et al. 1992; Piotto 1997; ANPA 2001). This means that their seeds attain moisture contents of 10.0% or less without losing viability (Villiers 1978). If viability is lost long before seed moisture reaches this level, then one concludes that the seeds are "recalcitrant" (Bonner 1996) which are always short-lived (Roberts 1973).

Species with recalcitrant seeds include several tropical plants (Arentz 1980; Farrant et al. 1993; Billia et al. 1999) as well as plants of the temperate zone, such as *Castanea, Quercus, Aesculus* and certain species of the genus *Acer* (Bonner 1990; McCreary and Koukoura 1990; Dickie et al. 1991; Bonner 1996; Connor and Bonner 2001). The storage behavior and longevity of some less known species, such as bay laurel, has not yet been investigated efficiently. The drying of bay laurel seeds causes a reduction of seed germination (Takos 2001; Takos et al. 2002; Tilki 2004; Sari et al. 2006). It is well known that recalcitrant seeds are short-lived and is difficult to store (Roberts 1973; Sun 1999). Not only the moisture content of the seeds, but also the rate of the desiccation has been shown to affect the viability of recalcitrant seeds significantly (Arentz 1980; Ramos et al. 1988).

The objectives of the present research were to examine the critical moisture content that produce a loss of seed viability of the *L. nobilis* seeds during desiccation at different drying rates and moisture levels, to determine the longevity of the seeds and to identify the most proper seed moisture content and the favorable storage conditions for improving longevity in storage.

Materials and methods

Collection

Mature fruits of *L. nobilis* were collected from trees located in Drama, Greece (altitude 90 m, latitude 41°10′, and longitude 24°09′) during early December. Determination of fruit maturity was based on pericarp color that turns dark black when ripe.

Determination of the (initial) moisture content of fresh fruits and seeds

The fruits were taken to the laboratory after collection. Seed moisture content was determined with the method and procedure proposed by ISTA (1999) for seeds. The determination was carried out in duplicate on two independently drawn working samples weighing 4–5 g, after the removal of the pericarp. Seeds were cut into pieces with a scalpel and after being weighed on an analytical balance of precision of three decimal places of a grammar, they were placed in an oven at 105 ± 1°C for 17 ± 1 h. Then they were weighed again and the seed moisture content was determined for each sample separately (Edwards 1987; Hartmann et al. 1997; ISTA 1999). The mean of the measurements, whose difference did not exceed 0.3–2.5%, constituted the seed moisture content, which was rounded off to the nearest 0.1% (ISTA 1999). Seed moisture content was expressed as a percentage of the fresh weight (%, f. wt). The same procedure was repeated for the fruits of *L. nobilis*.

Germination

Fresh, dried and stored fruits were subjected to pre-germination treatments which included soaking of the fruits in tap water for 10 days, as recommended by Takos (2001) in order to allow manual removal of the pericarp, with the water being changed every 2 days and cold stratification at 4 ± 1°C for 60 days in moist river sand which was previously sterilized at 150°C for 24 h, as described by Takos (2001). Following pre-germination treatment four replications of 100 seeds were used for germination. Germination occurred in glass Petri dishes, with each set of 100 seeds placed in one glass Petri dish of 20 cm in diameter. Seeds were pressed into the surface of the sand used as substratum, until half part of the seeds was being covered. Sand was remoisten when necessary to maintain moist conditions during germination. The Petri dishes were placed in a germination chamber for testing at a daily cycle of 25°C for 8 h with light intensity of 1,000 lux (light from cold light bulbs) and 20°C for 16 h in the dark. The first count was made on the 17th day, and continued under constant intervals of every 4 days until the end of the 10th week. Seeds were considered germinated when they exhibited 2 mm long radicals (ISTA 1999).

The germination value (GV) was determined for the drying rate experiment by using the equation GV = (PV) × (MDG). PV is the peak value defined as the quotient of the highest value of the cumulative germination percentage, divided by the number of days that took to reach the highest value since the beginning of the test. The "Mean Daily Germination" (MDG) is defined as the average daily germination (i.e., germination percentage divided by the number of days of the germination test (Czabator 1962).

First experiment: drying treatment

Fast drying

Seeds were separated into 23 lots, with each lot containing 400 seeds (4 × 100). Before placing the seeds in the oven, the fresh weight was taken for the set of 400 seeds. Then, each set (400 seeds) was placed in un-lidded Petri dish, and was dried in an air-flow oven at the temperature of 30°C (fast drying). To determine the desired seed moisture content (35.0, 30.0, 25.0, 20.0 and 15.0% on f. wt basis), each set was periodically weighed. On the 1st day, the sets of seeds were weighed on an hourly basis, while the following days they were checked three times a day (morning, afternoon and evening). After the sets of seeds reached the

desired moisture content, they were removed from the oven, and were subjected to pre-germination treatments described above, prior to the germination test.

Slow drying

Similarly to the fast drying, un-lidded Petri dishes with 400 seeds were placed on the laboratory bench under constantly alternating room conditions (slow drying), with mean day and night room temperatures of 25 and 15°C, respectively. Prior to any drying, the fresh weight of each set of 400 seeds was determined. Each sampling time (every 15 days), one set was weighted again and the moisture content (f. wt) was calculated. Then, those seeds were subjected to pre-germination treatments (similar to fast drying), and tested for germination as described above.

Second experiment: storage

For the storage experiment, two tests were conducted to determine the effect of specific storage conditions at 0 and 5°C on seed germination. The one test was conducted for seeds that were not subjected to any drying, and the other test included seeds that were subjected to partial drying.

No partial seed drying

For the first test, seeds that did not experience any drying were stored in the cooler under moist and dry conditions. Moist conditions were considered when placing the seeds in cotton bags that contained moist river sand (60% of water saturation) to allow partial rehydration of the seeds during storage. Dry conditions were determined by storing the seeds in airtight glass vials under the absence of moist sand, where no rehydration would take place under storage. Based on the above storage conditions, germination was determined at the end of the 4th, 8th and 12th month.

Partial seed drying

For the second storage test, seeds were partially dried to the desired moisture content of 30, 25 and 20% (on f. wt basis) as described above in the fast seed drying. The seeds were placed in the coolers (with temperatures of 0 ± 1°C, and 5 ± 1°C) in airtight vials. Under those storage conditions, germination was determined at the end of the 4th, 8th and 12th month.

Statistical analysis

The statistical analysis (ANOVA) was done with the help of the computer software package SPSS (Norusis 1997). The normality of the data was checked by using the Shapiro–Wilk test and homogeneity of variances among treatments with Levene's test of equality of variances (SPSS Inc. 1999). Some treatment groups presented significant deviations from normality and non-homogeneous variances as well. To remedy this problem, arcsin transformation was applied to the data and analysis of variance was done with the transformed values. The means were separated according to Duncan's test at the 0.05 level of probability (Mates 1994).

Results

Drying rate experiment

Moisture content

Under oven conditions, the desiccation rate was fast at the beginning, and it gradually reduced as time progressed (Table 1). Within only 7 h the moisture content dropped by 2% (37–35%) while 7% reduction (37–30%) in moisture content was achieved within 17 h. Additional reduction of the moisture content by 5% increments was achieved at slower rates as drying progressed. Reduction of 5% (30–25%) was achieved within 2 days, which was increased to 10 days (25–20%), and finally to 21 days (20–15%). Under constant alternating room temperatures seeds dried at a slower rate compared to the oven drying at 30°C (Table 1). Based on 15-day increments of slow drying, we observed a decrease in the rate that moisture content reduced. After 15 days drying there was an approximate 8% reduction in the moisture content that reduced to almost 5 (15–30 days), to 2.5 (30–45 days), and to 2% (45–60 days) for the subsequent 15-day increments of slow drying. Then the rate of moisture reduction was slowed down even further, until it stabilized at 12.8% moisture content after 150 days or more of drying.

Germination

Table 1 indicates significant differences among means, based on the effect of fast and slow drying treatment on seed germination. For both drying treatments, an initial reduction in seed moisture content significantly resulted in an increase in the percent of germination when compared to the initial germination (day 0). Specifically, for the fast drying treatment, the percent of germination increased from 55.1 (initial) to 81.0% (max germination) within 7 h. For the slow drying treatment, germination increased to 84.3% within 15 days that reached 90.9% (max germination) within 30 days of drying. As time progressed for both drying treatments, further reduction in the moisture content

Table 1 Bay laurel germination (%) and germination value after seed moisture content reduction

Drying rate	Desiccation days (h)	Seed moisture content (%, f. wt.)	Germination[a,c] (%)	Germination value (GV)[b,c]
	0 (initial)	37.0	55.1c	1.03B
Fast Oven at 30°C	(7 h)	35. 0	81.0a	9.55A
	(17 h)	30.0	44.0cd	1.36B
	3	25.0	33.2de	1.35B
	13	20.0	24.0e	0.18C
	34	15.0	1.1f	0.00D
Slow Room temp.	15	28.7	84.3a	7.17F
	30	23.5	90.9a	4.30E
	45	21.0	66.8b	0.96B
	60	19.6	49.4c	0.85B
	75	17.0	48.0c	0.32G
	90	15.2	11.0f	0.10C
	105	15.2	6.3f	0.03D
	120	15.3	2.1f	0.00D
	135	13.7	0.8f	0.00D
	150	12.8	0f	0.00D
	165	12.8	0f	0.00D
	180	12.8	0f	0.00D

[a] Percentages followed by different small letter are significantly different at $P = 0.05$, Duncan test

[b] Percentages followed by different capital letter are significantly different at $P = 0.05$, Duncan test

[c] Percentages are means of four replicates of 100 seeds. The values in the same columns were analyzed together

resulted to a substantial reduction in germination. For fast drying treatment, the percent of germination decreased to 24.0% with moisture content of 20%. For the slow drying treatment, the germination decreased to 11.0% when seed moisture was 15.2%. The percent of germination was dramatically reduced to 1.1% when moisture content was 15% in fast drying and to 11% or less when moisture content was equal or less than 15.2% in slow drying.

Initially, a partial drying resulted in an increase in the GV. For the fast drying rate, after a period of 7 h GV reached its maximum value of 9.55 with seeds containing 35% moisture content. For the slow drying treatment the maximum GV value (7.17) was achieved under a period of 15 days, with seeds retaining 28.7% moisture content.

Storage experiment

No partial seed drying

Statistical analysis indicated that storage duration under moist and dry conditions as well as storage temperature, significantly affected seed germination (Table 2). Seed storage under 0 ± 1°C indicated higher percent of germination when compared to the 5 ± 1°C, both under moist and dry storage conditions. Moist storage conditions had higher percent germination when compared to the dry conditions. Also, increase in storage time resulted to a substantial reduction in seed germination that reached zero values.

The highest percent germination (48%) was noted in the moist storage condition under 0 ± 1°C for the storage period of 4 months, although not significantly different from the control treatment (initial values). That percent of germination (48%) reduced to 36% and then to 11% after a period of 8 and 12 months of storage. Although not statistically different, dry storage conditions at the 0 ± 1°C indicated lower percent of germination when compared to the moist conditions. Under dry conditions, germination reduced to 41, 29, and 5% after a period of storage of 4, 8, and 12 months of storage, respectively. A substantial reduction on the percent of germination was indicated for the seeds that were stored under moist conditions at 5 ± 1°C, with germination being reduced to 31, 18 and 6% after a period of 4, 8 and 12 months, respectively. The lowest value on the percent germination was noted for seeds that were stored under dry storage conditions at 5 ± 1°C. Under these conditions, germination reached 4 and 0% after storage of 4 and 8 months, respectively.

Partial seed drying

Statistical analysis indicated that percent germination was significantly different among storage conditions when seeds were partially dried and stored at two temperatures regimes (0 ± 1 and 5 ± 1°C), and three storage periods (4, 8 and 12 months) (Table 3). Results revealed that after seeds were subjected to partial drying none of the above conditions resulted to an increased germination as high as that of the

Table 2 Bay laurel germination (%) and Germination value (GV) stored with the initial moisture content (37%)

Storage conditions	Storage duration (months)	Storage temperature			
		0 ± 1°C		5 ± 1°C	
		Germination[a,c] (%)	Germination value (GV)[b,c]	Germination[a,c] (%)	Germination value (GV)[b,c]
Control	0	55.1 a	1.03 AB	55.1 a	1.03 AB
Moist	4	48.0 ab	1.16 AB	31.0 cd	1.07 AB
	8	36.0 cd	0.22 C	18.0 e	0.05 C
	12	11.0 ef	0.19 C	6.0 fg	0.03 C
Dry	4	41.0 bc	1.50 A	4.0 fg	0.01 C
	8	29.0 d	0.15 C	0.0 g	0.00 C
	12	5.0 fg	0.02 C	0.0 g	0.00 C

[a] Percentages within columns followed by different small letter are significantly different at $P = 0.05$, Duncan test

[b] Percentages within columns followed by different capital letter are significantly different at $P = 0.05$, Duncan test

[c] Percentages are means of four replicates of 100 seeds

Table 3 Bay laurel germination (%) stored after seed moisture content reduction

Seed moisture content (% f. wt. basis)	Storage time (months)	Storage temperature			
		0 ± 1°C		5 ± 1°C	
		Germination[a,c] (%)	Germination value (GV)[b,c]	Germination[a,c] (%)	Germination value (GV)[b,c]
Initial moisture content (37)	0	55.1a	1.03 A	55.1a	1.03 A
30	4	41.0b	0.18B	36.0bc	0.08B
	8	30.0cd	0.07B	16.0e	0.18B
	12	8.0f	0.04B	1.0f	0.03B
25	4	32.0cd	0.18B	26.0d	0.10B
	8	16.0e	0.07B	7.0f	0.01B
	12	15.0e	0.00B	0.0f	0.00B
20	4	0.0f	0.00B	0.0f	0.00B
	8	0.0f	0.00B	0.0f	0.00B
	12	0.0f	0.00B	0.0f	0.00B

[a] Percentages within columns followed by different small letter are significantly different at $P = 0.05$, Duncan test

[b] Percentages within columns followed by different capital letter are significantly different at $P = 0.05$, Duncan test

[c] Percentages are means of four replicates of 100 seeds

control. The highest percent of germination (41%) was noted for seeds that were subjected to the least drying (30% moisture content) and were stored for a period of 4 months at 0 ± 1°C. Under the same storage conditions, the germination dropped to 30 and 8% after 8 and 12 months of storage, respectively. Under the same temperature conditions (0 ± 1°C), seeds with 25% moisture content indicated lower germination that reached 32, 16 and 15% when stored for a period of 4, 8 and 12 months, respectively. The high value of 15% after 12 months of storage possibly occurred, as an interaction of several factors or combinations and it requires further investigation.

Storage under 5 ± 1°C had a substantial reduction at germination. Specifically, seed with 30% moisture content under storage of 4 months had 36% germination and after 8 months of storage 16% germination, while for a storage period of 12 months the germination reduced to 1%. The results were even more profound when the seeds were partially dried to 25% moisture content, with germination dropping from 26 to 7 and to 0% for a storage period of 4, 8 and 12 months, respectively. The percent of germination reached zero when the seed moisture content reached levels lower to 20%, independently of temperature storage conditions (0 ± 1 and 5 ± 1°C).

Discussion

In the present study, a low reduction of the initial seed moisture content produced an increase in germinability, as it has been noted by Tilki (2004). The increase of this germinability may be due to the continuation of the embryo after-ripening process, which takes place during seed desiccation, whereas maturation drying is an essential part of seed development (Bewley and Black 1985). This is also referred for other recalcitrant species. For instance, Sycamore Maple (*Acer pseudoplatanus*), a species with recalcitrant seeds, displays a slight increase of its germinability after the partial desiccation of its seeds (Hong and Ellis 1990). The same phenomenon was observed on horse chestnut (*Aesculus hippocastanum*) recalcitrant seeds, where a brief period of desiccation increased germinability (Tompsett and Pritchard 1998). The phenomenon of germination increases as a result of low loss of seed moisture content after a brief period of desiccation which is also observed in species with recalcitrant and in species with orthodox seeds (Hong and Ellis 1990). The overall behavior of bay laurel seeds during the progressive reduction of their seed moisture content showed that their germinability was reduced when moisture was lost and that their germinability was fully preserved only within narrow high seed moisture content levels. The gradual reduction of the seed moisture content with both desiccation methods resulted in the accelerated reduction of the germination capacity to the point of nullification. The results of the experiments and of previous research demonstrated that the desiccation of seeds caused a reduction of seed germination (Takos 2001), proved that the bay laurel seeds are recalcitrant, because they cannot be dried to moisture content below 10.0% without losing viability (Roberts 1973; Bonner 1996). By desiccating bay laurel seeds, this research revealed the typical behavior of recalcitrant seeds. The "critical" minimum bay laurel seed moisture content that is necessary for the preservation of a low level of viability is approximately 15.0%, depending also on the desiccation method. The "critical moisture content" varies to the species (Farrant et al. 1988; Baskin and Baskin 2001). The corresponding "critical" moisture content for the recalcitrant seeds of other species is 25.0–30.0% for *Quercus* species (Bonner 1990), 20–50% for several tropical species (Bonner 1990), 20.0–22.0% for *Inga uruguensis* (Billia et al. 1999) and 10.0% for *Acer pseudoplatanus* (Dickie et al. 1991).

The results presented here suggest that seeds which were dried at room temperature showed higher germination percentage than those dried in the oven, for the same moisture content. It appears that the fast drying treatment has a more hazardous effect on seed germination of *L. nobilis* than the slow drying treatment. Tilki (2004) drying *L. nobilis* seeds at 20, 30 and 40°C reached similar conclusions for the same species. The rate of drying is very important as too rapid drying may lead to damage, and if the drying is too slow the chance of infection by pathogens is greatly increased (Mayer and Poljakoff-Mayer 1989).

As proved above, seed longevity was related to each storage conditions (dry or moist storage) as well as the storage temperature. The moisture content of the seeds played important role, too. Seeds stored at 0 ± 1°C remained viable for longer period compared with those stored at 5 ± 1°C. Cold moist storage in 0 ± 1°C with initial moisture content proved to be the most successful way of storage, whereas other various combinations of storage conditions (partial drying, temperature, duration and moisture) gave lower germination results. Results from this study demonstrate that there is a steep decrease in germination with the time. The longer the storage period was, the lower the germination exhibited by the seeds. Nevertheless, the seeds can be stored at 0 ± 1°C in moist conditions, with the initial seed moisture for 4–8 months and keep a satisfying germinability percentage. Seeds can be stored at the same temperature (0°C) even in dry conditions with the initial moisture content for 4 months. The moist conditions seem to be the most effective way of conserving recalcitrant seeds of *L. nobilis*. Moist storage is suggested for the tropical tree *Bertholletia excelsa* (Kainer et al. 1999). Cold-moist storage (0°C) is also suggested for *Quercus suber* (Anonymus 2000). The maintenance of germination after desiccation cannot be excluded as shown by our experiments. An important factor is the way of drying. Although we applied relative fast way of drying, maybe higher temperature and faster drying is more effective. Sun (1999) proved that fast drying of *Quercus rubra* seeds gave better results than slow drying. Research on *Q. suber* proved the same fact. The longer the drying period was, the lower the germination of the seeds.

Conclusion

1. Bay laurel seeds are recalcitrant.
2. A low reduction in the original seed moisture contents under fast or slow drying increased germination, while further progressive seed moisture content reduction produced a significant reduction of germinability.
3. The critical level of seed moisture content, the point at which seed viability becomes practically null, was determined to be equal to approximately 15.0% depending on the desiccation method.
4. The rate of the desiccation affects germination of *L. nobilis* L. seeds. Seeds dried at room temperature (slow rate) show higher germination than the ones dried in the oven (fast rate).
5. *Laurus nobilis* seeds do not maintain high viability in storage, like the rest of the recalcitrant seeds. However,

stored at 0 ± 1°C in moist conditions, without previous seed drying, they can maintain a satisfying germination percentage for 4–8 months.

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