International Journal of Natural and Engineering Sciences 2 (2): 91-95, 2008
ISSN: 1307-1149 www.nobel.gen.tr

Dormancy Depth, Prechilling, and Storability of Oriental Beechnuts (Fagus orientalis Lipsky)

Mustafa YILMAZ* Hüseyin DİRİK[2]

[1] Kahramanmaraş Sütçü İmam University, Faculty of Forestry, Kahramanmaraş / Turkey

[2] İstanbul University, Faculty of Forestry, İstanbul / Turkey

*Corresponding Author
e-mail: mustafayilmaz@ksu.edu.tr

Received: February 10, 2008
Accepted: March 24, 2008

Abstract

Pregermination during the prechilling process, dehydration sensitivity, and storability are the major problems of beechnut. The aim of this study was to develop an efficient prechilling technique for full dormancy removal and storability of Oriental beechnut from one provenance, Gölyaka. The tetrazolium test and cutting test on fresh non-stored beechnuts yielded relatively reliable estimation about germination capacity of Oriental beechnuts. Oriental beechnut from this provenance required about 11 weeks pre-chilling duration for full dormancy removal. Pregermination during the prechilling process was effectively avoided by prechilling at a controlled moisture content (about 30-32%) and enhanced storability was achieved by storing the Oriental beechnuts with reduced moisture content (about 6-8%) in sealed bags at –6ºC.

Key words: Beechnut, *Fagus orientalis*, Prechilling, Germination

INTRODUCTION

The majority of Oriental beech (*Fagus orientalis* Lipsky) forests, 1.751.483 ha, are in Turkey [1]. Due to degradation and poor management conditions, most of the Oriental beech forests of Turkey originate from sprouts rather than seeds. A good knowledge of seed material and handling is necessary to grow beech seedlings in a nursery environment and for successful natural regeneration from beechnuts. Beechnuts are particularly sensitive to the prechilling and storage conditions.

Mast years occur every 3-5 years in Oriental beechnuts [2], making the storage of beechnuts necessary. In the past beechnuts were considered sensitive to desiccation [3]. Studies on European beechnuts in 1960s and 1970s revealed that beechnuts could be dehydrated and stored in sealed containers at sub-zero temperatures for several years [4, 5, 6, 7]. Beechnuts are mostly classified as intermediate (sub-orthodox) in terms of storage and longevity [8, 9, 10].

Another problem with beechnut management is pregermination during cold-wet stratification in a medium such as sand. Beechnuts within the same provenance show different dormancy depth and some beechnuts germinate after shorter times while others remain dormant and fulfill their chilling requirements. Various studies revealed that beechnut (*F. sylvatica*) germination during prechilling could be avoided by controlling the moisture content (MC) of beechnuts [9, 11, 12, 13].

The purpose of this study was (1) to study the effects of controlled moisture content during prechilling, as well as prechilling duration, on the breaking of dormancy of Oriental beechnuts and (2) to examine the storability of Oriental beechnuts with low moisture content at low temperature. This study also aimed to compare the beechnut physiology of F. orientalis with that of other Fagus species.

MATERIAL AND METHODS

Oriental beechnuts were collected from Gölyaka-Düzce, (700-750 m; 40º 44' N; 31º 07' E), in the first week of November 2002. Beechnuts were air-dried at 18±1°C and empty and insect attacked beechnuts were removed. 1000-seed weight was measured on eight replicates of 100 beechnuts. Moisture content was determined by the low constant temperature oven method, 17 h at 104±1°C [14] and are given on fresh weight basis.

Germination Capacity: Germination tests were performed with four replicates of 50 beechnuts at 3°C [14] to determine germination capacity. The germinants were recorded once a week. The tests were terminated when two weeks passed without an observed germinant. Germination capacity was also assessed using the more rapid cutting test and tetrazolium test to identify sound and viable beechnuts, respectively. The cutting test was conducted by cutting the beechnuts longitudinally. The tetrazolium test used 1% 2,3,5-triphenyl tetrazolium chloride solution and the viability of embryos was evaluated according to ISTA [14] rules. Two hundred (4*50) beechnuts (cutting test) and beechnut embryos (tetrazolium test) were used.

Dormancy Depth and Prechilling at Controlled Moisture Content: Two prechilling periods (X and X+4) and three moisture content levels (28%, 32%, 35%) were used to determine the dormancy depth and appropriate moisture content level during the prechilling treatment. X duration is the number of weeks in which 10% of beechnuts germinate at 3°C [6]. Prechilling treatments were applied to break dormancy of beechnuts at three different controlled moisture content levels (28%, 32%, 35%) in the receptacles allowing air passage in the refrigerator (+3°C). The receptacles were weighed weekly to check for altered moisture content and distilled water was added by spraying if needed. The changes in the moisture content of the samples during the treatments were less than 1%.

The prechilled beechnuts were taken to germination test at 15°C, which is the optimum germination temperature for prechilled (nondormant) beechnuts [15, 16, 17]. The germination tests were carried out on two-layer filter paper in 15 cm diameter petri dishes with two hundred (4*50) beechnut samples. The petri dishes were checked every other day and germinants were counted and removed. The beechnuts were considered germinant when their radicles protruded 3 mm long and geotropism was observed. The germination tests ended on 28 d.

Beechnut Storage: For investigation of storability, beechnuts were stored at –6°C with two low moisture contents, 8% and 5.6%, for one year. The beechnuts were dehydrated to 8±0.1% and 5.6±0.1% moisture content in a climate cabinet (at 18°C with 20% relative humidity). After the storage period, germination tests were applied at +3°C.

Germination Parameters: In the germination tests, germination percentage (GP), mean germination time (MGT) and germination value (GV) were used as decisive factors. MGT was defined as the number of days or the number of weeks for the germination tests at 15°C and 3°C, respectively. GV were determined for the germination tests at 15°C. GP, MGT, and GV were calculated according to the following formulas:

$$GP(\%) = \frac{\sum n_i}{N} x100$$

Where GP(%) is the germination percentage, n_i is the number of germinating beechnuts on day of inspection, i, and N is the total number of incubated beechnuts for the test.

$$GV = \frac{\sum DGS}{N} \times (GP \times 10) \quad [18]$$

Where GV is the germination value, DGS is the daily germination speed, GP is the germination percentage, and N is number of days in which germination speed was counted (frequency).

$$MGT = \frac{\sum (t_i . n_i)}{\sum n_i} \quad [19]$$

Where MGT is the mean germination time, t_i is the number of weeks from the beginning of the test, and n_i is the number of germinating beechnuts recorded on week $t_{(i)}$.

GP, GV and MGT values of treatments were subjected to ANOVA to detect the significance of treatment means. Germination percentage was arcsine square root ($\sqrt{P}$) transformed to normalize error distribution prior to variance analyses. When a significant effect was detected, differences among the groups were identified using Duncan's New Multiple Range test.

RESULTS

Germination Capacity: 1000-seed weight was 251.5 g at 10% MC. The germination test at 3°C yielded 94.5% germination. The cutting test which exhibited the differences between sound and unhealthy beechnuts resulted 91.5% sound beechnuts (Figure1).

Figure 1. The result of cutting test: sound beechnuts (left) and the partly decayed unhealthy beechnuts (right).

There were significant differences between the germination test at 3°C and the tetrazolium test (Table 1). The tetrazolium test clearly showed dead tissues on the beechnut embryos (Figure 2). X duration was set at 7 weeks since the germination test at 3°C yielded about 10% GP on week 7.

Table 1. Comparison of germination test, cutting test and tetrazolium test.

Germination test	Cutting test	Tetrazolium test
Germination percentage (%)	**Percentage of sound beechnuts (%)**	**Percentage of viability (%)**
94.5 a	91.5 ab	86.5 b

The values followed by the same letter are not significantly different (P<0.05).

Figure 2. The results of tetrazolium test: sound beechnuts (left) and unhealthy beechnuts (right).

Dormancy Depth and Moisture Content: There was not a significant difference in germination capacity between beechnuts prechilled at different MC levels (Table 1). Fungal contamination was observed during the final weeks of prechilling treatment at 32% and 35% MC.

In contrast, the effect of prechilling duration on GP, MGT, and GV was evident. Lengthening of prechilling by four weeks, from X to X+4, increased GP from 31.83% to 83% and accelerated MGT from 13.67 days to 7.66 days.

Germination time increased with increasing moisture content of the beechnuts during the prechilling. In the prechilling treatment for X+4 weeks, mean germination time of beechnuts prechilled at 35% MC was significantly lower (faster) than those of 28% and 32% (Table 2). Similarly, the germination value of 35% MC was significantly higher than those of 28% and 32%.

Table 2. The effects of MC and prechilling durations on GP, MGT, and GV. (Germination temperature of 15°C).

Prechilling Duration	MC During the Prechilling	GP (%)		MGT (d)		GV	
X	28%	30.0[b]	31.83	14.85[c]	13.67	3.13[c]	3.94
	32%	35.0[b]		13.25[c]		4.83[c]	
	35%	30.5[b]		12.92[c]		3.87[c]	
X+4	28%	85.5[a]	83.00	8.44[b]	7.66	41.55[b]	43.87
	32%	81.0[a]		8.02[b]		40.34[b]	
	35%	82.5[a]		6.52[a]		49.71[a]	

The values in the same column followed by the same letter are not significantly different (P<0.05).

After one year of storage at –6°C with 5.6% and 8% MC, germination capacity did not vary significantly and the initial high germination percentage (94.5%) was preserved. The stored beechnuts did however germinate faster than fresh beechnuts (Table 3, Figure 3). MGT of the stored beechnuts were found 1.4 week (about 10 days) lower (faster) than that of the fresh beechnuts.

Table 3. Germination percentage and mean germination time of Oriental beechnuts before and after one year of storage at –6°C.

	Germination percentage (%)	Mean Germination Time (week)
Before storage	94.5[a]	9.41[b]
One-year storage at 5.6% MC	94.5[a]	7.96[a]
One-year storage at 8% MC	92.0[a]	8.06[a]

Results on the same column followed by the same letter are not significantly different (P<0.05).

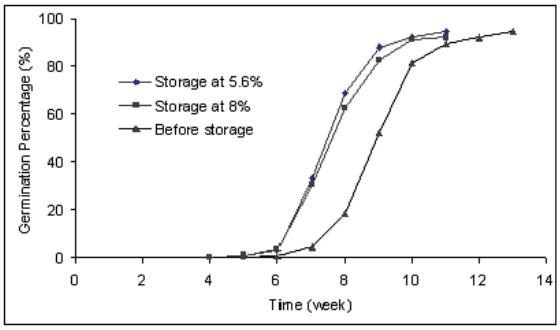

Figure 3. Germination percentage of fresh and one-year stored beechnuts at +3°C.

DISCUSSION

The germination capacity of beechnuts is normally determined by incubating the non-dormant beechnuts at 3°C [14] for about 12-18 weeks. Both dormancy breaking and germination occur in germination tests at 3°C. Quick viability tests such as the cutting test and tetrazolium test are commonly applied to beechnuts to estimate the germination capacity in a short time because of its deep dormancy [20, 21].

The result of the cutting test (91.5% sound beechnuts) is very close to the values of the germination tests (94.5%) at 3°C and tetrazolium test (86.5% viable beechnuts). It is also observed that cutting the beechnuts at high moisture content, about 30-35%, is easier and the cut surface of the embryo is bright that makes the interpretation of viability easier (Figure 1). The tetrazolium test revealed significantly lower viability than the germination test at 3°C since some beechnuts with necrotic areas or partial color, usually considered non-viable [14], germinated at 3°C. Likewise necrotic areas and local darkening were observed at cotyledons of some germinated beechnuts when they were cut.

"X duration" represents the period in which beechnuts reach about 10% germination at 3°C. The definition of "X duration" was introduced due to heterogeneity of beechnut dormancy and is broadly accepted [6, 12, 20, 17]. The use of "X duration" allowed better assessments of prechilling requirements, and effects of altering variables on germination. In this study, there were great differences between X and X+4 prechilling durations (Table 2). X and X+2 prechilling durations were also insufficient for full dormancy removal in European beechnuts [22, 9].

In traditional cold-wet stratifications, treatments are terminated when some radicles protrude. This study indicates that only one third of beechnuts (about 30-35%) are able to germinate at 15°C in X duration which is a similar time to the traditional cold-wet stratification length. That the non-dormant seeds wait for the still dormant seeds during the prechilling treatment is one of the most advantageous aspects in the method of prechilling without media at controlled moisture content.

Suszka [6] suggested that moisture content of European beechnuts should be greater than 26% during the prechilling in order to achieve dormancy-breaking. 31% MC was suggested as the optimum MC for prechilling without media [23]. In applying this method for Oriental beechnuts, the moisture content between 28% and 32% appeared to be optimal, which is in agreement with the recommendation of Suszka [6] of 31% for European beechnuts. Some unexpected pregerminations were observed during the prechilling treatments at 35% MC. Moisture content of 34% or above increases the vulnerability of the tips of radicles when the beechnuts are dried back after prechilling [9, 13]. Gosling [8] reports that the maximum MC of European beechnuts and the threshold MC at which germination occurs are 41% and 36%, respectively. Similar results were observed in Oriental beechnuts except that 35% MC appeared to be sufficient for germination of prechilled Oriental beechnuts.

Beechnut deterioration and beechnut aging vary with storage conditions, especially temperature and humidity (24, 25). In a recent study on the storage of beechnuts of *Fagus sylvatica* and *Fagus crenata*, 7.8-11.5% MC and –10/-20°C temperature were suggested [26]. Poulsen [27], on the other hand, stated that MC of beechnuts should be reduced to 5% for 10 or more years storage, and that 7% MC was acceptable for 3-4 years storage. In this study, there was not a significant decrease in the germination capacity of Oriental beechnuts during one year

storage at 8% and 5.6% MC, which gives an impression of long time storability like the other beech species' beechnuts. Drying of beechnuts shortens the prechilling durations about 2-4 weeks [16, 13]. In this study, the dried-stored beechnuts germinated more quickly and there was about 10 days difference between MGT of the fresh beechnuts and dried-stored beechnuts (Table 3).

Considering the germination characteristics and prechilling and storage conditions, Oriental beechnuts have very similar physiological characteristics to European beechnuts. That the mast years are more frequent in Oriental beech is an advantage for its storage duration compared to European beechnuts. The collection of Oriental beechnut in a mast year with storage for a period of 3-4 years would provide sufficient beechnuts until the next mast year.

This study shows that the best way of dormancy breaking of Oriental beechnuts is the method of prechilling without media which provides full dormancy breaking for all beechnuts by extending the prechilling duration without pregermination. This method also gives practitioners flexibility at nurseries during the sowing season since the treatment could be extended several weeks without pregermination occuring. Current research also demonstrates the storability of dehydrated Oriental beechnuts at negative temperature, -6ºC. Further detailed research on the optimum MC during storage, the optimum storage temperature, and storage longevity should be conducted.

REFERENCES

[1] OGM. 2007. Orman Atlası, Ankara, 86p.

[2] Tosun S. 1992. Bolu Yöresi Doğu Kayını (Fagus orientalis Lipsky.) Ormanlarında Tohum Verimi Üzerine Araştırmalar. OAE Yay. Tek. Bul. No: 232, Ankara, 75 p.

[3] Saatçioğlu F, Ürgenç S. 1960. Doğu Kayını (Fagus orientalis Lipsky.) tohumlarının çimlendirilmesinde soğuk ıslak işlemin etkileri üzerine araştırmalar, Orman Fak. Derg. Seri A, X(2): 7-26.

[4] Suszka B. 1975. Cold storage of already after-ripened beech (Fagus sylvatica L.). Arboretum Kornickie 20: 299-315.

[5] Bonnet-Masimbert M, Muller C. 1975. La Conservation des faînes est possible. Revue Forestière Française, XXVII (2): 129-137.

[6] Suszka B. 1979. Seedling emergence of beech (Fagus sylvatica L.) seed pretreated by chilling without any medium at controlled hydration level. Arboretum Kornickie, 24:111-135.

[7] Muller C, Bonnet-Masimbert, M. 1980. Long term storage of beechnuts: results of large scale trials. In: Wang, B.S.P. & Pitel, J.A. (ed.) Proceedings of International Symposium on Forest Tree Seed Storage. Petawawa-Canada, CFS, p. 178-203.

[8] Gosling PG. 1991. Beechnut storage: a review and practical interpretation of the scientific literature. Forestry, 64:51-59.

[9] Derkx MPM, Joustra MK. 1997. Dormancy breaking and short-term storage of pre-treated Fagus sylvatica seeds. In: R.H. Ellis, Murdoch, A.J. & Hong, T.D. (ed.) Basic and Applied Aspects of Seed Biology. Kluwer Ac. Pub., Dordrecht. p. 269-278.

[10] Pukacka S, Hoffmann SK, Goslar J, Pukacki PM, Wójkiewicz E. 2003. Water and lipid relations in beech (Fagus sylvatica L.) seeds and its effect on storage behaviour. Biochemica et Biophysica Acta, 1921:48-56.

[11] Suszka B, Zieta L. 1977. A new preserving treatment for cold-stored beech (Fagus sylvatica L.) seed. Arboretum Kornickie 22: 237-255.

[12] Muller C, Bonnet-Masimbert M. 1989. Breaking dormancy before storage: an improvement to processing of beechnuts (Fagus sylvatica L.). SST 17, 15-26.

[13] Muller C, Laroppe E, Bonnet-Masimbert M. 1999. Further developments in the redrying and storage of prechilled beechnuts (Fagus sylvatica L.): Effect of Seed Moisture Content and Prechilling Duration. Annals of Forestry Science 56: 49-57.

[14] ISTA. 1999. International rules for seed testing. Rules 1996, Seed Science and Tech. 27 suppl., 333 p.

[15] Suszka B. 1974. Storage of beech (Fagus sylvatica L.) seeds for up to 5 winters. Arboretum Kornickie 19: 105-128.

[16] Thomsen KA. 1997. The effect of harvest time and drying on dormancy and storability in beechnuts. In: Basic and Applied Aspects of Seed Biology. Proceedings of The Fifth International Workshop On Seeds, Reading 1995, 45-51.

[17] Yılmaz M. 2005. Doğu kayını (*Fagus orientalis* Lipsky.) tohumlarının fizyolojisi üzerine araştırmalar [The Researches On The Physiology of Oriental Beechnuts (*Fagus orientalis* Lipsky.)] PhD Thesis, Istanbul University, Institute of Science, 170p.

[18] Djavanshir K, Pourbeik H. 1976: Germination value-a new formula. Silvae Genetica 25:79-83.

[19] Bewley JD, Black M. 1994. Seeds: Physiology of Development and Germination. Plenum Press, New York, 445p.

[20] Suszka B, Muller C, Bonnet-Masimbert M. 1996. Seeds of Forest Broadleaves, From Harvest to Sowing. INRA, France, 295 p.

[21] Yılmaz M. 2006. Doğu Kayını (*Fagus orientalis* Lipsky.) Tohumlarında Ekim Öncesi İşlemler. Orman Mühendisliği Dergisi, Sayı: 7-8-9: 27-30.

[23] Suszka B, Kluczyńska A. 1980. Seedling emergence of stored beech (Fagus sylvatica L.) seed chilled without medium at a controlled hydration level and pregerminated in cold-moist conditions. Arboretum Kornickie, 25:231-255.

[24] Ratajczak, E. Pukacka, S. 2005. Decrease in beech (Fagus sylvatica) seed viability caused by temperature and humidity conditions as related to membrane damage

and lipid composition. Acta Physiologiae Plantarum 27, 3–12.

[25] Pukacka, S. Ratajczak, E. 2007. Age-related biochemical changes during storage of beech (Fagus sylvatica L.) seeds. Seed Science Research, 17:45-53.

[26] León-Lobos P. Ellis RH. 2002. Seed storage behaviour of Fagus sylvatica and Fagus crenata. Seed Sci. Res. 12:31-37.

[27] Poulsen KM. 1993. Predicting the storage life of beechnuts. Seed Sci. and Tech. 21(2): 327-337.