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# SEED STORAGE AND CRYOEXPOSURE BEHAVIOR IN HAZELNUT (*CORYLUS AVELLANA* L. CV. BARCELONA)

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## SUMMARY

Moisture contents of individual hazelnut (*Corylus avellana* L. cv. Barcelona) seed and seed parts were determined before and during storage at room temperature. The moisture content and percentage radicle emergence were determined after each storage period and after desiccation over silica gel prior to liquid nitrogen exposure. Hazelnut seeds had a high coefficient of variation for moisture content. No loss of germination was observed for seeds desiccated down to 5% moisture (fresh wt. basis). Although desiccated whole seeds did not survive $LN_2$ exposure, the embryonic axes excised after cryoexposure of whole seeds survived and were grown in tissue culture.

KEY WORDS : Hazelnut, seed, storage, cryopreservation, embryonic axes, *Corylus*.

## INTRODUCTION

Hazelnut seeds are reported to be recalcitrant (1), however Bonner (2) considered them suborthodox. Stanwood (3) classified hazelnut seeds as "desiccation-tolerant, $LN_2$-sensitive" and suggested that more extensive research is needed to further characterize their behavior during the $LN_2$ cooling/rewarming cycle. Currently hazelnut seed cannot be stored for more than one year without substantial loss of viability (4), a characteristic of recalcitrant seeds. Moisture content of individual recalcitrant seeds may vary considerably, so their coefficient of variation is large as compared to a similar sample of orthodox seeds (5). This study investigated the variability of hazelnut seed moisture content, the effect of storage on moisture content and seed survival following storage and cryoexposure.

## MATERIALS AND METHODS

Hazelnuts (*Corylus avellana* L. cv. Barcelona) were obtained at the beginning of harvest season on September 18, 1993 from Wayne Chambers, Albany, Oregon.

### *Variability in moisture content of Hazelnut seeds*

Hazelnuts were manually cracked to remove the pericarp. The testa attached to the seed was not removed unless it came out with the pericarp. Fifty seeds were divided into cotyledons and embryonic axes and weighed in aluminum foil boats. Seed parts were dried in an oven at 103$^{o}$C for 16 hr. and the dry weights were taken after cooling in a desiccator. Moisture content was expressed as a percentage of fresh weight. Percentage composition of axes and cotyledons were determined on a dry weight basis. The coefficient of variability of moisture content, a relative measure of the variation in moisture content present in the seed sample, was calculated as (100 x standard deviation of the mean moisture content)/mean moisture content.

### *Storage of hazelnut seeds at ambient temperature*

Nuts were stored in 70 liter burlap bags at ambient temperature (20$^{o}$C) and relative humidity (20-40%). Seed moisture content and percentage germination were assessed monthly. The moisture content was determined by drying seeds at 103$^{o}$C for 16 hr. and expressed as a percentage of fresh weight. Four replicates of 10 seeds were used for moisture content determination, and three replicates of 25 seeds were used for germination tests.

For germination, the nuts were cracked to remove some or all of the pericarp and soaked in a solution of $GA_3$ (10 mg/l) for 24 hr. The radicle emergence (RE) test was performed between papers (6) in a seed germinator with alternating temperatures of 10$^{o}$C (14 hr.)/25$^{o}$C (10 hr.) under 12 hr. photoperiod. Radicle emergence (minimum 1 mm) was determined after four weeks. Seeds without RE but which were not moldy were tested for viability using the tetrazolium test (2,3,5-triphenyltetrazolium chloride, 1.0%) (6).

### *Desiccation and cryoexposure of hazelnut seeds*

Whole nuts (with pericarp) were dried over 125 g silica gel for 0, 16, 24, 32, and 48 hr. Silica gel was changed after every period of desiccation. After each desiccation period, 190 nuts were removed for moisture determination, cryoexposure and germination (control, not cryoexposed). Three replicates of 25 nuts each were used in cryoexposure and germination tests and four replicates of 10 nuts each were used for moisture content determination.

For cryoexposure, each replicate of nuts was placed into a separate compartment of a storage rack and immersed directly in liquid nitrogen for 16-24 hr. The nuts were then removed from the cryotank, thawed at room temperature, the pericarp cracked, and placed in trays for germination. Percentage RE of control and cryoexposed seeds and moisture content determination were carried out as described in the storage experiment.

*Survival of embryonic axes after cryoexposure of hazelnut seeds*

Sixty whole nuts were cryoexposed by direct plunging into liquid nitrogen. The nuts were stored for about one hr. in liquid nitrogen then thawed at room temperature. Thirty nuts were cracked open and a tetrazolium test was performed to determine the viability of the seeds after cryoexposure. Another 30 nuts were cracked, the embryonic axes excised (10 axes per replicate), sterilized, soaked in $GA_3$ (10 mg/100 ml) for 5 minutes and cultured on a basal NCGR-COR medium (7). Thirty control nuts (not cryoexposed) were cracked and the axes cultured as above. Another 30 control axes were excised and tested for moisture content. This experiment was repeated at monthly intervals for 6 months with nuts stored at $20^{o}$C.

## RESULTS AND DISCUSSION

*Variability in seed composition and moisture content*

Hazelnut seeds were composed mainly of cotyledons (95.9% by weight) with only a small percentage (0.2%) accounted for by the embryonic axis (Table 1). Generally in recalcitrant seeds, embryonic axes account for 0.25% of the dry weight of whole seed compared to an average of 1.4% for orthodox seeds (8). The moisture content of the cotyledons was more variable than that of the embryonic axes. The embryonic axes had and average moisture content of 35% while the cotyledons averaged 29% ; however, these values were not significantly different. Variability in moisture content among whole seeds was also high. In recalcitrant seeds such as *Avicennia* (9), *Hevea brasiliensis* (10), *Nephelium lappaceum* and *Artocarpus heterophyllus* (8) variability in moisture content is observed among seeds within the same batch. A higher moisture content in the embryonic axis than in other seed parts is also a characteristic of *Hevea* seeds (10).

Table 1. Percentage composition (dry weight), moisture content and coefficient of variability of moisture content of hazelnut seed parts at harvest on September 18.

| | % Composition[a] (dry weight) | % Moisture content[b] | Moisture content coefficient of variability(%) |
|---|---|---|---|
| Whole seed (Without pericarp) | 100 ± 0.0 | 28.7 ± 6.6 | |
| Cotyledons | 95.9 ± 3.7 | 29.0 ± 7.2 | |
| Embryonic axis | 0.2 ± 0.03 | 35.3 ± 7.9 | |

[a] n = 50

[b] Mean ± standard deviation

*Storage of seeds at ambient temperature*

Freshly harvested seeds displayed high viability (68% RE + 24% TTZ positive) which decreased with storage to 4% RE and 12% TTZ positive at six months (Figure 1). A rapid decrease in moisture from 25% to 9% was observed after one month of storage: by six months the moisture had decreased to 4%.

Fungal and bacterial contamination of seeds during germination was a problem and increased throughout the storage period from 5% at harvest to 80% after five months. Fungal/bacterial growth could be reduced by drying the seeds to a low moisture content or soaking them in a fungicide before storage, as was done for *Hevea* (11). Hazelnut seeds that were free from contamination, but whose radicles did not emerge, were still viable as shown by positive results with the tetrazolium test (Figure 2). Repeated $GA_3$ treatments are often needed to stimulate germination in hazelnut seeds due to their irregular germination pattern (12).

The low RE of fresh seeds (68%) and seeds stored for one month (51%) is due to fungal contamination (5% and 24%) as well as to the onset of seed dormancy after harvest. Hazelnuts are known to have dormancy induced during the first month of dry storage (13). Bradbeer (14) has suggested that primary dormancy is induced by inhibitors which occur mainly in the testa and pericarp and that dry storage imposes a deeper (secondary) state of dormancy.

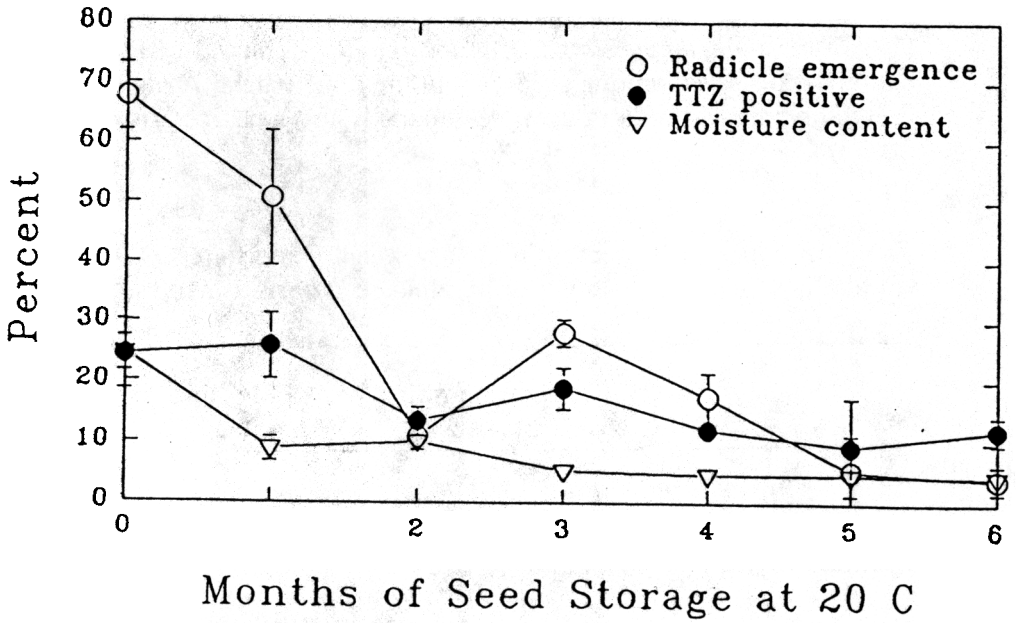


Figure 1. Percentage of radicle emergence (RE), tetrazolium (TTZ) positive for non-RE seeds, and moisture content of 'Barcelona' hazelnut seeds after storage at ambient temperature ($20^{o}$C) for up to six months.

Table 2. Percentage of radicle emergence (RE) of hazelnut seeds following cryoexposure in liquid nitrogen at various initial moisture contents following si'ica gel desiccation.

| Duration of desiccation Control[b] (hr.) | Whole seed moisture content (%)[a] | % RE Cryoexposed | |
|---|---|---|---|
| 0 | 24.6 ± 2.9 | 0 | 67.6 ± 6.6 |
| 16 | 18.5 ± 1.0 | 0 | 64.5 ± 5 |
| 24 | 19.7 ± .6 | 0 | 63.6 ± 5.0 |
| 32 | 14.6 ± 2.2 | 0 | 68.8 ± 10.4 |
| 48 | 16. ± 2.3 | 0 | 56.0 ± 10.6 |

[a] mean ± standard deviation
[b] dried but not exposed to $LN_2$.

*Desiccation and cryoexposure of hazelnut seeds*

Moisture content of hazelnut seeds during desiccation over silica gel for 0 to 48 hr. declined from about 25% to 16% (Table 2). The germination percentage of the control (dried but not exposed to $LN_2$) did not show much change due to desiccation, indicating that specific moisture contents are not critical for germination. Seeds did not survive cryoexposure either with or without desiccation. Moisture content of hazelnut seeds plateaued at about 15%, well above the 4-8% required for many seeds for survival following cryoexposure (3). The moisture content of desiccated whole hazelnut seeds is comparable to embryonic axes of rubber (15), jackfruit (16) and coffee (17) that survived $LN_2$ exposure.

Direct immersion into liquid nitrogen may have caused differential cooling rates within the seeds. Visual inspection of thawed seeds showed extensive damage to cotyledonary tissue. Lack of germination after cryoexposure was probably due to this damage to the cotyledons, thus depriving the embryonic axes of the nutrients necessary to develop into plants and resulting in axis death. Stanwood (3) indicated that solidification of oils occurs at temperatures below - 40°C and this may be related to seed damage associated with $LN_2$ cooling of hazelnut seeds. Axes isolated from the frozen seeds survived at or near 100% if the axis moisture content was below 8% (Figure 2). The phenomenon of embryonic axis survival after cryoexposure of seeds is observed in some palm species (18).

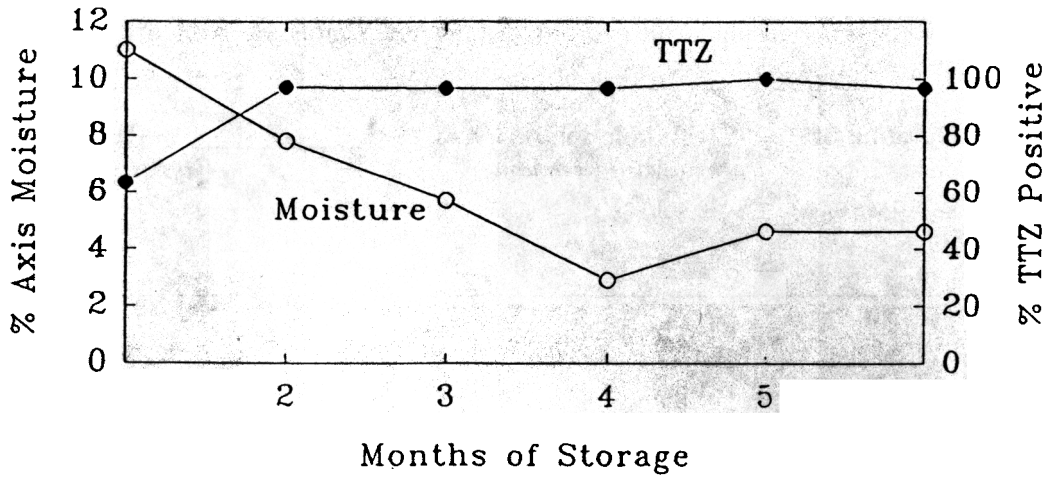


Figure 2. Moisture content of axes isolated from hazelnuts and TTZ viability of axes following cryoexposure of the whole seed.

Hazelnuts do not fully satisfy the definition of recalcitrant seed storage behavior since they survive considerably greater desiccation than is typically observed in recalcitrant seeds. However, the proportion of the total seed dry weight for cotyledons and embryos follows that of recalcitrant seeds, as does the high coefficient of variation of seed moisture content. The large variation in moisture content in hazelnut was reflected in the variability of survival following drying or cryoexposure. Whole hazelnut seeds are sensitive to $LN_2$ even when dried to 15% moisture. Further studies are necessary to determine if hazelnut seeds should be placed into an intermediate category as suggested for coffee by Ellis et al.(20).

*Survival of axes after cryoexposure of seeds*

The moisture content of axes decreased from the original fresh seed level of 35% to 4% after six months of storage (Figure 2). Embryonic axes with as low as 3% moisture content had 97% viability after cryoexposure. Shoot and root formation decreased for both the control and the cryoexposed axes with increased storage time at 20°C (Figure 3). Viability of the axes remained high for five months of storage and root production was moderate. Shoot production on control axes was initially 100%, decreased to about 35% at one month and continued to decrease to less than 10% after six months of seed storage at 20°C. This decline in shoot production and a similar effect on radicle growth was probably due to dormancy induced in the axes during dry storage (13). Two-weeks of seed stratification overcame this inhibition and resulted in shoot growth in tissue-cultured isolated-embryonic axes from stored seed (19). Preliminary whole-seed cryoexposure tests with stratified seed produced no viable axes (data not shown).

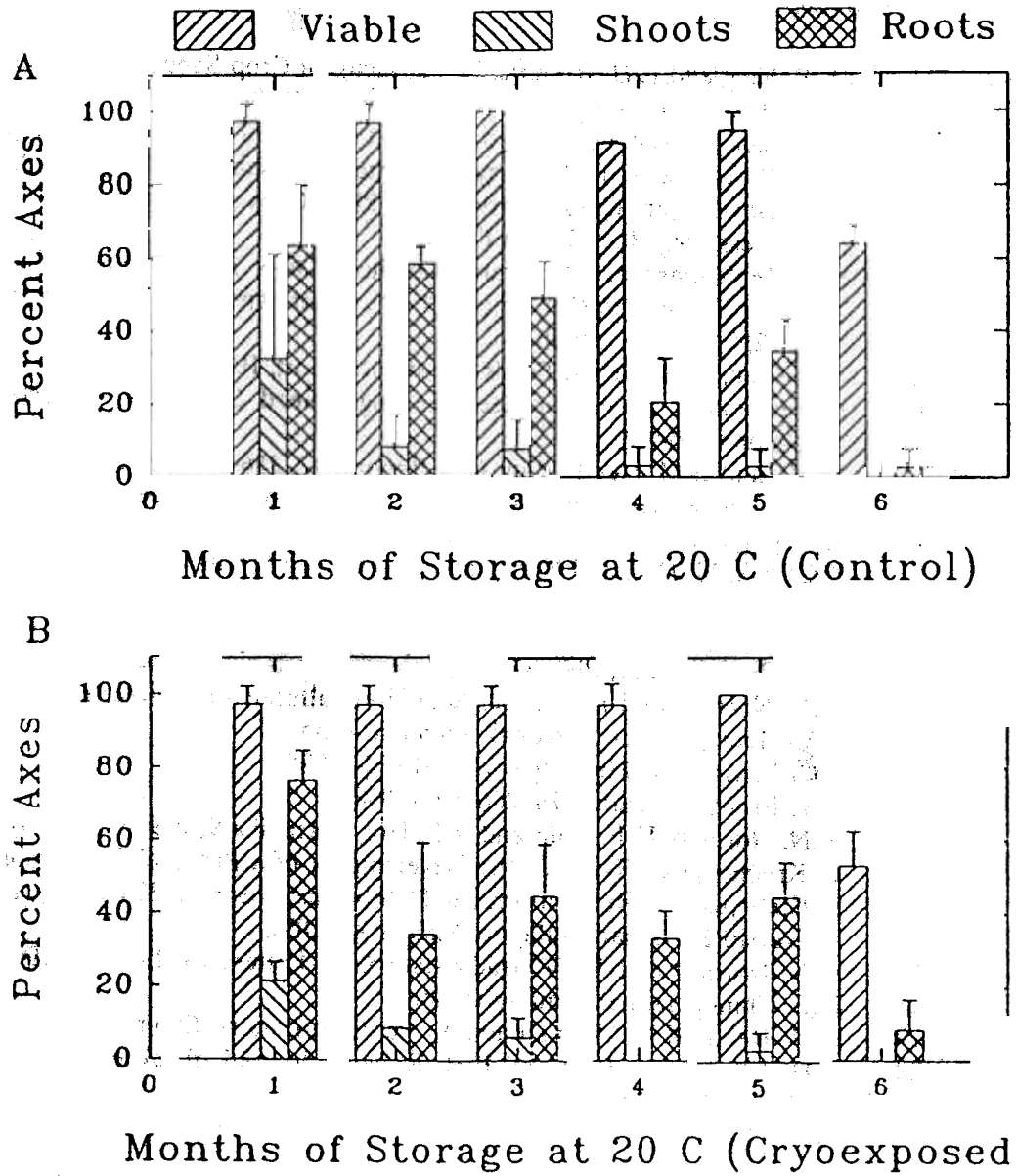


Figure 3. Viability, shoot and root production of isolated embryonic axes excised from control (A) or cryoexposed (B) whole hazelnut seeds.

## CONCLUSIONS

Embryonic axes of hazelnut seeds at very low moisture contents survived cryoexposure when the whole seeds were frozen. Freezing whole hazelnuts (dried to 15% moisture) in liquid nitrogen may be a useful method of germplasm storage. The axes could be excised and cultured after thawing of seeds. This technique would avoid the problems of cotyledon damage and subsequent contamination of whole seeds during germination, but may be complicated by decreased shoot production in axes from stored seed. Improvements in stratification techniques may make storage of hazelnut seeds possible.

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## REFERENCES


1. M.W. King and E.H. Roberts, in "Recalcitrant Crop Seeds", H.F. Chin and E.H. Roberts, eds. Tropical Press, Kuala Lumpur, (1980).
2. F. Bonner, in "Technologies to Maintain Biological Diversity" vol. 2. part D, pp. 630-672. Office of Technology Assessment, Washington D.C. (1986).
3. P.C. Stanwood, in "Cryopreservation of Plant Cells and Organs", K.K. Kartha, ed. CRC Press Inc., Boca Raton (1985).
4. S.A. Mehlenbacher, Hazelnuts (Corylus). Chap. 17 in: "Genetic Resources of Temperate Fruit and Nut crops 11," J.N. Moore and J.R. Ballington Jr. eds. Acta Hort. 290, 791-836 (1990).
5. H.F. Chin, Recalcitrant Seeds - A status report, IBPGR (1988).
6. International Rules for Seed Testing. Seed Science and Technology 21, supplement (1993).
7. X. Yu and B.M. Reed, Plant Cell Reports 12, 256-259 (1993).
8. H.F. Chin, B. Krishnapillay and P.C. Stanwood, In "Seed Moisture". CSSA Special publication no. 14 (1989).
9. P. Berjak, M. Dini and N.W. Pammenter, Seed Science and Technology 12, 365-384 (1984).
10. M.N. Normah, Effects of Temperature on Rubber (*Hevea brasiliensis* Muell. - Arg.) Seed Storage, PhD Thesis, Universiti Pertanian Malaysia, Malaysia (1987).
11. M.N. Normah and H.F. Chin, Acta Horticulturae 153, 258-259 (1989).
12. S.A. Mehlenbacher, personal communication.
13 B.C. Jarvis, New Phytology 75, 491-494 (1975).
14. J.W. Bradbeer, Planta 78, 266-276 (1968).
15. M.N. Normah, H.F. Chin and Y.L. Hor, Pertanika 9, 299-303 (1986).
16. B. Krishnapillay, Towards the Development of a Protocol for Cryopreservation of Embryos of a Recalcitrant Seed (*Artocarpus heterophyllus* Lam.), PhD Thesis, Universiti Pertanian Malaysia, Malaysia (1989).
17. M.N. Normah and M. Vengadasalam, Cryo-Letters 13, 199-208 (1992).
18. H.F. Chin, personal communication.
19. B.M. Reed, M.N. Normah and X. Yu, Cryo-Letters, Submitted.
20. R.H. Ellis, T.D. Hong and E.H. Roberts, Journal of Experimental Botany 41 (230), 1167-1174 (1990).