Annals of Botany **86**: 1017–1021, 2000
doi:10.1006/anbo.2000.1265, available online at http://www.idealibrary.com on IDEAL®

Storage Behaviour of *Salix alba* and *Salix matsudana* Seeds

H. L. MARODER*, I. A. PREGO, G. R. FACCIUTO and S. B. MALDONADO

Instituto de Recursos Biológicos, Instituto Nacional de Tecnología Agropecuaria (INTA), Las Cabañas y Los Reseros s/n, CP 1712, Castelar, Argentina

Received: 25 May 2000 Returned for revision: 23 June 2000 Accepted: 27 July 2000

Effects of dehydration, storage temperature and humidification on germination of *Salix alba* and *S. matsudana* seeds were studied. Newly released seeds showed 100 % germination before and after dehydration to 11–12 % moisture content. Germination of the high vigour lot (100 % initial normal germination) was not affected by dehydration to 6·7 % moisture content but germination decreased with further dehydration to 4·3 %. The lower vigour lot (75 % initial normal germination) was more susceptible to dehydration and germination decreased following dehydration to 6·7 % moisture content. Dry seeds of both species survived immersion in liquid nitrogen without loss of viability. The germination of seeds stored with 9 % moisture content decreased to 35–40 % in 5 months at −20°C or in 2 months at 5°C. However, at 25°C seeds entirely lost viability within 2 weeks. Seeds showed improved performance when stored at −70°C > − 20°C > 5°C > 25°C and tolerated dehydration to a moisture content in equilibrium with 15 % relative humidity. Results suggest that they are orthodox in storage behaviour although they are short-lived. Humidification treatment of low vigour seed lots resulted in a remarkable increase in germination percentage.

Key words: *Salix alba*, *Salix matsudana*, willow, seed storage behaviour, dehydration, humidification, cryopreservation.

INTRODUCTION

Willow (*Salix* spp.) seeds are short-lived. Seed viability is lost within a few days at room temperature (Teng and Yu, 1948; Arya *et al.*, 1988) and because of this rapid loss in viability, commercial seeds are not available (Brinkman, 1974). Willow seeds were provisionally classified as recalcitrant (King and Roberts, 1979; Pence, 1995) although recently Hong *et al.* (1996) classified seeds from 28 species of *Salix* as orthodox, except for *S. caprea* which was intermediate. Hong *et al.* (1996) took into account that seeds of the 28 species had been maintained without loss in viability for some years at subzero temperature and low moisture content (Crocker, 1938; Sato, 1955; Zasada and Densmore, 1977, 1980; Simak, 1982; Densmore and Zasada, 1983; Bol'shakow, 1988). *S. alba* and *S. matsudana* were not included in the re-classification. The objective of this paper is to describe physiological characteristics of both *Salix alba* and *S. matsudana* seeds in relation to their storage behaviour.

MATERIALS AND METHODS

Plant material

Seeds from trees of *Salix alba* L. and *S. matsudana* Koidz grown in Delta area, Provincia de Buenos Aires, Argentina, were collected during mid-October 1997 using the following procedure. Branches carrying catkins were separated from trees once the capsules turned yellowish and the seeds had begun to be released. The catkins were immediately separated from branches, spread on trays and maintained for 12 h at room temperature (approx. 24°C). Every hour during the 12 h period, seeds released from capsules were collected and removed from the surrounding cotton with compressed air. The seeds were dried initially for 3 h in ambient conditions and then stored at 5°C. Seed moisture content was approx. 40 % (wet basis) immediately after release and was reduced to 11–12 % after 3 h in ambient conditions. Moisture content (mc) of two samples of 100 seeds was determined gravimetrically by the oven method (1 h at 130°C ± 1°C) and calculated on a wet weight basis (wb) (ISTA, 1999). Seeds were tested for germination on top of three pieces of filter paper moistened with 3·5 cm^3 distilled-deionized water in 6-cm-diameter Petri dishes at 25°C ± 1°C and 16 h fluorescent light/8 h dark for 6 d. Four replicates of 25 seeds were used for each treatment. Normal germination was evaluated. In some experiments abnormal germination and non-germinated seeds were also assessed in addition to normal germination. Germination was considered normal if the seedling developed cotyledons, hypocotyl and root, and was erect; seedlings not meeting these criteria were classified as abnormal. Although fresh seed germinated in 12 to 24 h (up to 72 h for low vigour seed), definitive counting was carried out on the sixth day after sowing in order to recognize abnormal seedlings without doubt.

Dehydration of seeds and liquid nitrogen treatment

Salix alba seed lots with 100 and 75 % germination and 12 % mc were dehydrated to different moisture contents.

* For correspondence. Fax 54 11 4481 2360, e-mail hmaroder@cirn.inta.gov.ar

0305-7364/00/111017+05 $35.00/00

Seeds were held at 5°C for 24 h in an atmosphere equilibrated with saturated solutions of K_2CO_3 (approx. 43 % relative humidity, RH) and LiCl (approx. 15 % RH) or with concentrated H_2SO_4 (approx. 1 % RH). Moisture contents after dehydration were 9 % (K_2CO_3), 6·7 % (LiCl) and 4·3 % (H_2SO_4). Thereafter seeds were placed in cryovials and immersed in liquid nitrogen (LN) for 1 h; one sample with 100 % germination and 12 % mc was maintained in LN for 11 months. Immediately after LN treatment, cryovials with seeds were transferred to a water bath at 36°C for 1 min. The effect of treatments on germination was evaluated. A similar procedure was followed with a *S. matsudana* seed lot having 100 % germination and 11 % mc. Dehydration was carried out only over LiCl-saturated solution and concentrated H_2SO_4. Moisture contents after dehydration were 6·5 % (LiCl) and 4·4 % (H_2SO_4).

Storage at different temperatures

A *Salix alba* seed lot with 75 % germination and 9 % mc was stored at −20, 5 and 25°C. In another experiment the *S. matsudana* seed lot with 100 % germination and 10·5 % mc was placed at −70°C. Seeds were stored in sealed containers. Periodically samples were removed from storage and tested for germination (as above). For the −70°C stored lot, germination at 24 h from sowing was included as a vigour indicator; in this case the germination criterion was hypocotyl protrusion (unlike other seeds, the first visible sign of germination of *Salix* seeds is the extension of the hypocotyl; Pólya, 1961).

Humidification treatment

The effect of humidification on the germination of *S. alba* and *S. matsudana* seeds was tested. Three *S. alba* seed lots (9 % mc), two with approx. 43 % and one with approx. 16 % germination, and two seed lots of *S. matsudana* (10·5 % mc) with 90 and 46 % germination were used. Seeds were maintained in a saturated atmosphere (100 % RH): *S. alba* at 25°C and *S. matsudana* at 17°C. Samples were removed at 3 h intervals and tested for germination. In seed samples of *S. matsudana*, moisture content and germination percentage were also determined hourly during the first 3 h of humidification.

Results were analysed statistically using analysis of variance. The differences between treatment means were determined with Tukey's test.

RESULTS AND DISCUSSION

Immediately after release from catkins, *S. alba* seeds had 40 % moisture content and showed 100 % germination; immersion of newly released seeds in liquid nitrogen was lethal (data not shown). Three hours drying in ambient conditions lowered the moisture content to 12 % without reducing seed germination. Seeds dried this way were not affected by liquid nitrogen treatment (Table 1). It appears that 3 h drying was enough to lower the seed moisture content below that of freezable water (Stanwood, 1985).

The seed lot with 100 % germination tolerated desiccation to 9 and 6·7 % moisture content using potassium carbonate and lithium chloride as desiccants which provided approx. 43 and 15 % RH, respectively (Table 1). However, further dehydration to 4·3 % by sulphuric acid, which provided about 1 % RH, reduced germination approx. four-fold. The seed lot with 75 % initial germination was more susceptible to dehydration. Although germination at 9 % moisture content was slightly reduced, further dehydration to 6·7 and 4·3 % moisture content reduced germination significantly to 55 and 33 %, respectively (Table 1). Germination of seeds dried between 4·3 and 12 % moisture content was not affected by liquid nitrogen treatment in either high or low viability lots.

Similar results with respect to dehydration and tolerance to liquid nitrogen were obtained with *S. matsudana* seeds (Fig. 1). Newly released seeds (41 % moisture content) dried for 3 h in ambient conditions after release (11 % moisture content) showed 98 and 100 % germination, respectively. Germination was slightly reduced by dehydration at 6·5 % moisture content (lithium chloride) (from 100 to 86 %), while a considerable reduction to 16·5 % germination at 4·4 % moisture content (sulphuric acid) was observed. Seeds died when exposed to liquid nitrogen immediately after release from their capsules, but seeds that survived dehydration treatments (at 11, 6·5 and 4·4 % moisture content) remained alive after exposure to a cryogenic temperature. Moreover, the *S. matsudana* seed lot at 11 % moisture content and stored for 11 months in liquid nitrogen showed no significant decrease in germination (data not shown). Taking into account the tolerance to liquid nitrogen, cryogenic storage would be a suitable method for long-term conservation of *S. matsudana*, as suggested by Pritchard and Seaton (1993) for species with short-lived seeds. Additionally, the very small size of *Salix* seeds results in an effective cryogenic procedure which is easy to operate.

The effect of storage temperature on viability of *S. alba* seeds is shown in Fig. 2. In accordance with the reported short life of *Salix* seeds, viability was lost in 2 weeks at 25°C (Fig. 2). Seed survival was extended for several weeks at 5°C and for several months at −20°C. The *S. matsudana* seed

TABLE 1. *Influence of moisture content on normal germination (%) of two seed lots of* Salix alba *that differ in initial germination exposed (+LN) or not to liquid nitrogen (−LN)*

Moisture content (%, wb)	Initial normal germination			
	100 %		75 %	
	−LN	+LN	−LN	+LN
12	100[a]	98[a]	75[a]	70[a]
9	94[a]	96[a]	68[a]	66[a]
6·7	89[a]	93[a]	55[b]	54[b]
4·3	23[b]	28[b]	33[c]	36[c]

Values with the same superscript within columns are not significantly different at $P = 0{\cdot}05$ by Tukey test.

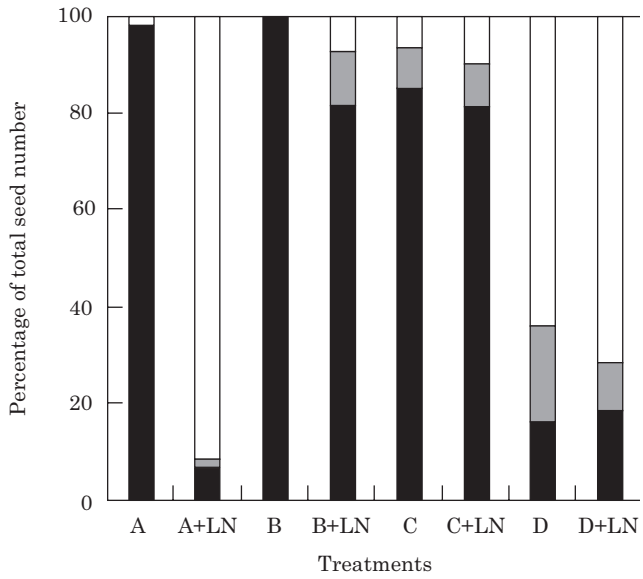

FIG. 1. Germination (%) of *S. matsudana* seeds after dehydration treatments, each with and without immediate immersion in liquid nitrogen (LN) for 1 h. A, Newly released seeds (control): 40 % mc; B, 3 h after seed release at ambient conditions: 11 % mc; C, desiccated over LiCl saturated solution: 6·5 % mc; D, desiccated over concentrated H_2SO_4: 4·4 % mc. Bar values are means of four replicates of 25 seeds. (■), Normal germ; (▩), abnormal germ; (□), non-germ.

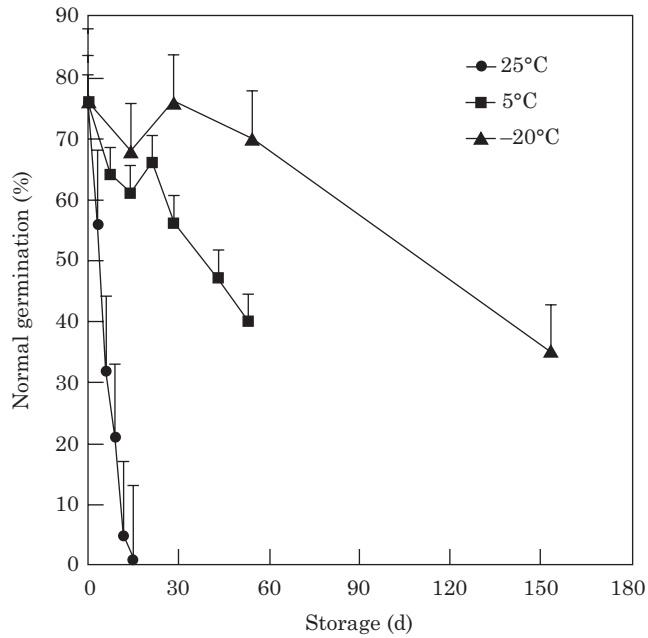

FIG. 2. Germination (%) of *Salix alba* seeds after storage at 9 % moisture content and 25, 5 or −20°C. Values are means of four replicates of 25 seeds ± s.e.

lot with 10·5 % moisture content stored at −70°C maintained initial germination levels for up to 30 months (Table 2).

Our results showed that seeds of *Salix alba* and *S. matsudana* tolerated desiccation to 6·7 and 6·5 % moisture contents, respectively, i.e. in equilibrium with about 15 % RH (over lithium chloride) at 5°C, and that longevity was greater with lower storage temperature. According to the criteria for orthodox seeds (Hong and Ellis, 1996), we conclude that seeds of the two species investigated showed orthodox seed storage behaviour. This conclusion is in accordance with the orthodox seed storage behaviour of 28 other *Salix* species classified by Hong *et al.* (1996).

A relatively long duration of storage of willow seeds from different species has been reported at sub-zero temperatures (Sato, 1955; Zasada and Densmore, 1980). For example, seeds of *Salix alaxensis* Coville, *S. glauca* Linn., *S. bebbiana* Sarg. and *S. novae-angliae* Anderss. maintained a high percentage germination during 2 years' storage at −10°C. Similarly, Sato (1955) reported that the viability of *S. urbaniana* Seemen seeds, maintained at −8°C for 535 d, ranged from 20 to 60 % depending upon the desiccation treatment. However, differences in species, growth region, seed lot quality and moisture content preclude any strict comparison of results.

TABLE 2. *Germination (%) of* S. matsudana *seeds after storage for different periods at 10·5 % moisture content and −70°C*

Months of storage	Germination (%)		
	24 h*	Normal + Abnormal	Normal
6	95 ± 1·8	100	98 ± 1·4
18	96 ± 4·5	100	95 ± 3·9
24	100	100	97 ± 4·5
30	89 ± 9·6	99 ± 3·3	97 ± 4·5

* From sowing.
Values are means of four replicates of 25 seeds ± s.e.

Humidification treatment in the deteriorated seed lots of *S. alba* (Fig. 3) and *S. matsudana* (Fig. 4) resulted in a reduction, followed by a rise in germination percentage. For example in lot A of *S. alba* (Fig. 3), a decrease from 42 % initial germination to 33 % occurred after the first 3 h of treatment. After humidification treatment for 6 h, germination reached 41 %, thus recovering the initial germination level, and then continued to increase up to 60 %, more than the initial value. Because of the shortage of seeds of lots B and C, full recovery of germination was not achieved. Humidified *S. matsudana* seeds from lots A and B (Fig. 4) followed a similar pattern. A three-fold reduction in germination percentage (normal germination) was observed after 3 h humidification: from 90 and 46 % initial germination to 30 and 15 % for lots A and B, respectively. Beneficial effects of humidification on seed germination in various species have previously been reported and attributed to the avoidance of imbibition damage (Bewley and Black, 1985; Ellis *et al.*, 1990). In seeds of one Salicaceae species, *Populus alba*, Pólya (1961) detected an increase in germination percentage from 47 to 65 % in seeds previously humidified to 12·6 % moisture content. However, in our experiments, a decrease, rather than an increase, was

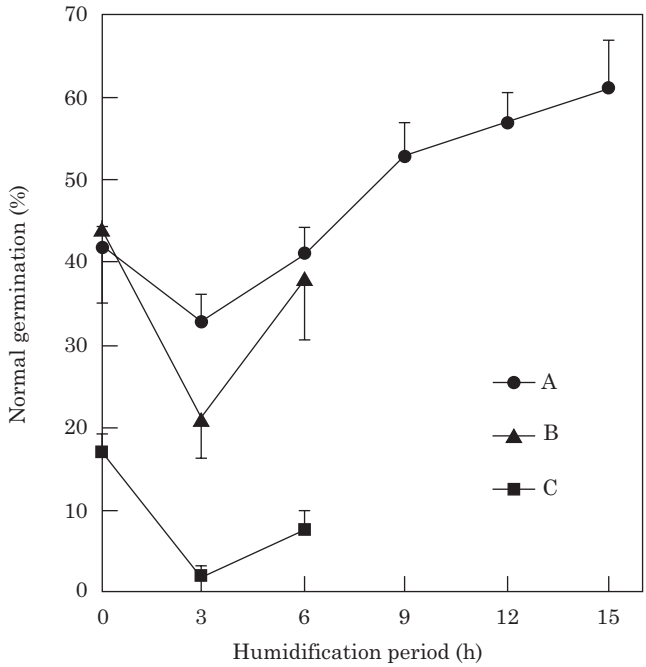

FIG. 3. Effect of seed humidification at 100 % RH and 25°C on germination (%) of *Salix alba* seed lots (A–C) with different initial germination. Initial seed water content was 9 %. Values are means of four replicates of 25 seeds ± s.e.

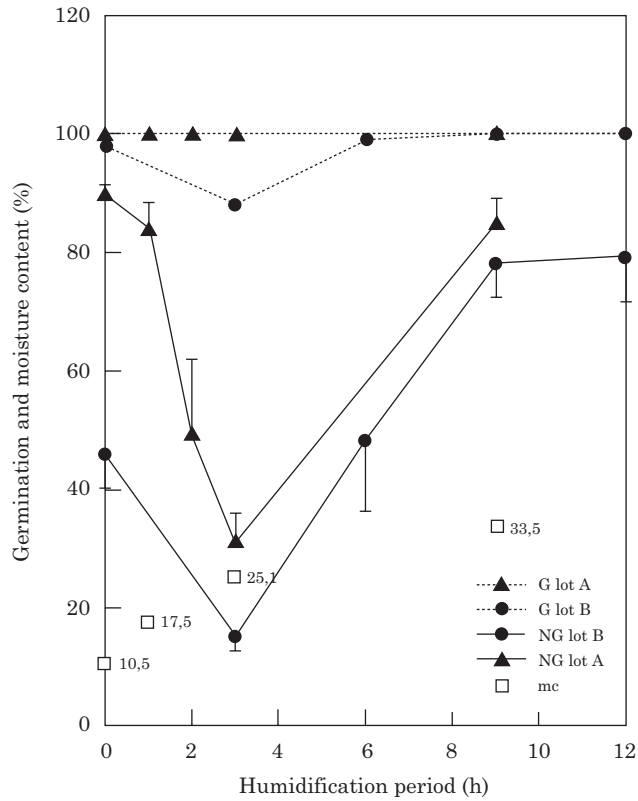

FIG. 4. Effect of seed humidification at 100 % RH and 17°C on germination (%) of *Salix matsudana* seed lots (A and B) with different initial germination. Initial seed water content was 10·5 %. Normal + abnormal germination (G), normal germination (NG). Values are means of four replicates of 25 seeds ± s.e.

promoted by 3 h humidification in the two Salicaceae species studied. At this time percentage germination was the lowest and moisture content averaged 25·1 % (Fig. 4), a level that should preclude any imbibition damage (Hong and Ellis, 1996). Even after humidification for 1 h, seeds of lot B with 17·3 % moisture content showed some loss in germination percentage. However, several germination tests made with *S. alba* and *S. matsudana* seed lots having moisture contents between 9 and 12 % showed 98–100 % normal germination when assayed without previous humidification, suggesting that imbibition damage had not occurred (data not shown). It may be that deteriorated seed lots could show a different response. Thus, germination at shorter humidification periods (less than 3 h) remains to be evaluated for low vigour lots.

The initial decrease in germination percentage of *S. matsudana* seed lots was followed by a remarkable increase, from 15 and 31 % for lots A and B, respectively, to approx. 80 % for both lots after 9 h humidification. Curiously, in most seed samples from both lots A and B, germination (normal plus abnormal germination) reached 100 % along the entire humidification treatment (Fig. 4). A decrease only occurred in the more deteriorated seed lot B at 3 h humidification and corresponded to the lowest normal germination percentage.

Results suggest that some deteriorative process other than imbibition damage accounts for the observed decrease in germination in the first hours of the humidification treatment. Figure 4 shows that seedling growth was much more affected than germination ability by this apparent deterioration. From these results it seems likely that metabolic repair must be involved in the beneficial effects of humidification on seed recovery that followed the initial decay (Burgass and Powell, 1984; Bray *et al.*, 1989; Gray and Drew, 1991; Fujikura *et al.*, 1992). Such germination behaviour of humidified *S. alba* and *S. matsudana* seeds has not been reported previously for any Salicaceae species.

ACKNOWLEDGEMENTS

The authors thank Dr T. D. Hong for reviewing the manuscript and providing constructive criticism. Funding support from SECYT (BID 802/OC-AR-PICT 02353) is acknowledged.

LITERATURE CITED

Arya SR, Bhagat S, Virendra-Singh I. 1988. Preliminary studies on seed germination and viability of *Salix alba* and *Salix elegans*. *Van-Vigyan* **26**: 88–90.

Bewley JD, Black M. 1985. *Seeds. Physiology of development and germination*. New York: Plenum Press.

Bol'shakow NM. 1988. Some information on the dispatch, storage and viability of seed of *Chosenia arbutifolia*. *Byulleten'Glavnogo Botanicheskogo Sada* **180**: 69–72 (*Seed Abstracts* 1992, 15, N° 3292).

Bray CM, Davison PA, Ashraf M, Taylor RM. 1989. Biochemical changes during priming of leek seeds. *Annals of Botany* **63**: 185–193.

Brinkman KA. 1974. Seeds of woody plants in the United States. *Agriculture Handbook* N° 450. Forest Service U.S. Department of Agriculture.

Burgass RW, Powell AA. 1984. Evidence for repair processes in the invigoration of seeds by hydration. *Annals of Botany* **53**: 753–757.

Crocker W. 1938. Life-span of seeds. *Botanical Review* **4**: 235–274.

Densmore R, Zasada JC. 1983. Seed dispersal and dormancy patterns in northern willows: ecological and evolutionary significance. *Canadian Journal of Botany* **61**: 3207–3216.

Ellis RH, Hong TD, Roberts EH. 1990. Effect of moisture content and method of rehydration on the susceptibility of pea seeds to imbibition damage. *Seed Science and Technology* **18**: 131–137.

Fujikura Y, Kraak LH, Basva SA, Karssen CM. 1992. Comparison of the effects on germination of osmopriming and hydropriming in cauliflower seeds. In: Côme D, Corbineau F, eds. *Fourth International Workshop on Seeds. Basic and Applied Aspects of Seed Biology, Angers, France*, *Volume 3*, *Paris: Université Pierre et Marie Curie 1993*, 1021–1025.

Gray D, Drew RLK. 1991. Comparison of polyethyleneglycol polymers betaine and L-proline for priming vegetable seed. *Seed Science and Technology* **19**: 581–590.

Hong TD, Ellis RH. 1996. A protocol to determine seed storage behaviour. In: Engels JMM, Toll J, eds. *IPGRI technical bulletin 1*. Rome: International Plant Genetic Resources Institute.

Hong TD, Linington S, Ellis RH. 1996. *Seed storage behaviour: a compendium* (Electronic version). Rome: International Plant Genetic Resource Institute.

International Seed Testing Association. 1999. International rules for seed testing. *Seed Science and Technology* **27**: Supplement, Rules.

King MW, Roberts EH. 1979. *The storage of recalcitrant seeds*. Rome: International Board for Plant Genetic Resources.

Pence VC. 1995. Cryopreservation of recalcitrant seed. In: Bajaj YPS, ed. *Biotechnology in agriculture and forestry*. Berlin: Springer-Verlag, 29–50.

Pólya L. 1961. Injury by soaking of *Populus alba* seeds. *Nature* **189**: 159–160.

Pritchard HW, Seaton PT. 1993. Orchid seed storage: historical perspective, current status, and future prospects for long-term conservation. *Selbyana* **14**: 89–104.

Sato Y. 1955. On the viability of Salicaceae seeds. *Research Bulletins of the College of Experiment Forests, Hokkaido University* **17**: 225–266.

Simak M. 1982. Germination and storage of *Salix caprea* L. and *Populus tremula* L. seeds. In: Wang BSP, Pitel JA, eds. *Proceedings of the International Symposium of Forest Tree Seed Storage, Petawawa/Canada. Ottawa, Canada: Environment Canada, Canada Forestry Service*, 142–160.

Stanwood PC. 1985. Cryopreservation of seed germplasm for genetic conservation. In: Kartha KK, ed. *Cryopreservation of plant cells and organs*. Florida: CRC Press, Inc., 199–226.

Teng SC, Yu H. 1948. Propagation of weeping willow from seed. *Botanical Bulletin Academia Sinica* **2**: 131–132.

Zasada JC, Densmore R. 1977. Changes in Salicaceae seed viability during storage. *Seed Science and Technology* **5**: 509–517.

Zasada JC, Densmore R. 1980. Alaskan willow and balsam poplar seed viability after 3 years storage. *Tree Planter's Notes* **31**: 9–10.