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Priority Science for the Preservation of Priority Crops

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The International Treaty on Plant Genetic Resources for Food and Agriculture identifies 64 crops or crop complexes for conservation and sustainable use. Although many can be banked conventionally as seed, lifespans will vary. For seeds that are short-lived, and the 10 species requiring explant recovery in vitro, cryobiotechnology is required for their preservation.

This contains what is known about the long-term *ex situ* preservation potential of the world's major crops as delineated by Annex I of the ITPGRFA. Whilst *in vitro* storage is not considered here, recovery growth *in vitro* is an important step in cryopreservation protocols for explants of those species that do not produce seeds or for which the retention of genetic homogeneity is essential.

Seed Viability Constants: quantify the effects of moisture content and temperature on orthodox seed lifespan (for reviews, see Roberts and Ellis, 1989; Pritchard and Dickie, 2003) and are available for 19 species from 17 genera out of a total of 76 genera (Table 1). The genera are: *Beta, Brassica, Cicer, Eleusine, Helianthus, Hordeum, Malus, Oryza, Pennisetum, Phaseolus, Phleum, Pisum, Sorghum, Trifolium, Triticum, Vigna* and *Zea*. As this coverage of genera represents only 22% of the total (i.e., 17 / 76) in Annex I, it is recommended that more, detailed studies of seed longevity sensitivity to water and temperature are undertaken on a wider range of agrobiodiversity, as the viability constants form the basis of predictions of longevity in agricultural seed (gene) banks across the world.

Longevity Estimates: of these 'easy-to-store' seeds, either based on the viability constants or on half-lives (P50s), indicates that not all seeds are long-lived (Table 1). Apart from *Rhaphanus* and *Sinapis*, with P50s around the 100 y mark, some other genera in the *Brassica* complex have estimated half-lives about 25 y. Interestingly, when using non-conventional banking conditions (-5 to -10°C, and ultra-dried with silica gel) seeds of many genera in this complex, e.g. *Barbarea, Brassica* and *Sinapis*, survive well for 40 y. Only eight of 45 species for which long-term P50 estimates of lifespan in cold storage are available have values ~ 100 years or more: *Agrostis stolonifera, Avena sativa, Lens culinaris, Lolium temulentum, Medicago sativa, Pisum sativum, Vigna radiata* and *Raphanus sativus.* This represents only 17% of the sampled species. As shorter seed lifespans may be a feature of accessions in the world's gene banks, cryopreservation needs further exploration as a means of improving longevity, even for orthodox seeds (Li and Pritchard, 2009). There can be considerable variation in longevity performance for the same species in different genebanks. This probably relates to seed lot differences and to the use of slightly different methodology. A review of this variability would be helpful in redefining gene bank standards.

Cryobiotechnology: It is the combination of ultra-low temperature storage and explant recovery *in vitro*, is required for 10 of the 64 crop and crop complexes: breadfruit (*Artocarpus*), citrus (*Citrus*), coconut (*Cocus*), major aroids (*Colocasia, Xanthostoma*), yams (*Dioscorea*), sweet potato (*Ipomoea*), apple (*Malus*), cassava (*Manihot*), banana and plantain (*Musa*) and potato (some *Solanum*) (Table 1). For breadfruit and coconut the challenge is that the seeds produced are recalcitrant (desiccation sensitive), and across the genus *Citrus* seed desiccation tolerance is variable. Nonetheless, some progress has been made on the cryopreservation of embryos and embryonic axes of these species. Some of the other species in this cluster may need to be maintained clonally and shoot tip cryopreservation has often been used successfully, particularly employing vitrification or droplet vitrification. Post-cryo survival of shoot tips can be relatively low (<40%) in *Cocus* (coconut) and *Ipomoea* (sweet potato), indicating the need for further protocol development. Some of these species also produce 'true' seed (e.g. cassava, apple) that are orthodox and could be cryopreserved. For

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Table 1. *Ex situ* preservation of priority crops (Annex I, ITPGRFA). Examples from the literature are given as a starting point to other reading. When material for storage is highlighted in bold, there is a need for recovery growth after cryopreservation *in vitro*

Crop	Genus	Material for storage	Example of preservation*
Foods			
Breadfruit	*Artocarpus*	**embryo axis**	Vitrification/cryopreservation of embryonic axes of *Artocarpus heterophyllus* with 50% developing into plants (Thammasiri, 1999). No reliable method apparent for *Artocarpus altilis* embryonic axis cryopreservation.
Asparagus	*Asparagus*	seed	Survival of *A. officinalis* seed known over c. 5 y storage under room or cool conditions (RBG Kew, 2016)
Oat	*Avena*	seed	P50: *A. sativa* = 117 y (Walters *et al*., 2005).
Beet	*Beta*	seed	Seed viability constants: *B. vulgaris:* Ke 8.943; Cw 4.723; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016)
Brassica complex	*Brassica, etc*	seed	[Includes species in: *Brassica, Armoracia, Barbarea, Camelina, Crambe, Diplotaxis, Eruca, Isatis, Lepidium, Raphanobrassica, Raphanus, Rorippa, Sinapis*]. P50s: *Brassica juncea, B. napus, B. oleracea* = 23-59 y; *Crambe abyssinica* = 21 y; *Isatis tinctoria* = 27 y; *Lepidium sativum* = 26 y; *Raphanus sativus* = 120 y; *Sinapis alba* P50 = 76 y (Walters *et al.,* 2005) Seed viability constants: *Brassica juncea,* Ke 7.768; Cw 4.56; Ch 0.0329; Cq 0.000478. *Brassica napus*, Ke 7.718; Cw 4.54; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016). Ultra-dry seeds of many species of Brassicaceae survived after almost 40 years of storage at -5 to -10°C, for example in the genera *Barbarea, Brassica, Sinapis* (Pérez-García *et al*., 2007).
Pigeon pea	*Cajanus*	seed	No problem for *C. cajan* seed storage under international standard seed bank conditions (RBG Kew, 2016).
Chickpea	*Cicer*	seed	P50: *C. arietinum* = 70 y (Walters *et al*., 2005) Seed viability constants: *C. arietinum*, Ke 8.502; Cw 4.602; Ch 0.295; Cq 0.000491 (RBG Kew, 2016)
Citrus	*Citrus*	**embryo, embryo axis** (+ decoated seed)	Partially dried explants (seeds, zygotic embryos, embryonic axes) have been cryopreserved for at least 17 species (See Malik *et al*., 2012). Citrus shoot apices, cell suspensions and somatic embryos have also been successfully cryopreserved.
Coconut	*Cocos*	**embryo, shoot tip**	Zygotic embryos from different cultivars were cryopreserved without inducing gross morphological, genetic or epigenetic changes in the recovered plants (Susunandar *et al*., 2010). Shoot tips cryopreserved by encapsulation-dehydration had 20% regrowth into leafy shoots 8 months after cryopreservation (N'Nan *et al*., 2008).
Major aroids	*Colocasia, Xanthosoma*	**shoot tip**	[Includes taro, cocoyam, dasheen, tannia]. Droplet vitrification cryopreservation of *in vitro* shoot tips of taro (*Colocasia*), with post-thaw regeneration of 73-100% (Sant *et al*., 2008). Cocoyam is more difficult to cryopreserve. Few studies on *Xanthosoma*.
Carrot	*Daucus*	seed	P50: *D. carota* = 30 y (Walters *et al*., 2005)
Yams	*Dioscorea*	**shoot tip**	Following cryopreservation by a modified droplet vitrification technique, 52% of surviving explants developed further within 1 month (Leunufna and Keller, 2003).
Finger millet	*Eleusine*	seed	Seed viability constants: *E. coracana*, Ke 9.508; Cw 5.08; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016).
Strawberry	*Fragaria*	seed	*F. virginiana* seed had 75 % viability after pre-drying to 15 % RH and storage for 107 days at -20°C (RBG Kew, 2016).
Sunflower	*Helianthus*	seed	P50: *H. annuus* = 50 y (Walters *et al*., 2005) Seed viability constants: *H. annuus*, Ke 6.74; Cw 4.16; Ch 0.039; Cq 0.000478 (RBG Kew, 2016).
Sweet potato	*Ipomoea*	**shoot tip**	Shoot tips of 24 sweet potato accessions had shoot formation levels of 2 – 66% after droplet vitrification cryopreservation (Vollmer *et al*., 2014).
Barley	*Hordeum*	seed	Seed viability constants: *H. vulgare*, Ke 9.144; Cw 5.342; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016).
Grass pea	*Lathyrus*	seed	P50: *L. odoratus* = 40 y (Walters *et al*., 2005)
Lentil	*Lens*	seed	P50: *L. culinaris* = 365 y (Walters *et al*., 2005)
Apple	*Malus*	**dormant bud, shoot tip** (+ seed)	Of 77 accessions (31 species and hybrids) in the Malus collection, 37 had recovery >40 % (69%) when winter vegetative-buds were cryopreserved (Höfer, 2015). *In vitro* shoot tips from four *Malus* species had 57% recovery after encapsulation-dehydration cryopreservation (Li *et al*., 2014). Seed viability constants: *M. pumila*, Ke 7.316; Cw 4.119; Ch 0.04; Cq 0.000428 (RBG Kew, 2016)

Contd.

Crop	Genus	Material for storage	Example of preservation*
Cassava	*Manihot*	**shoot tip** (+ seed)	*In vitro* shoot tips of cassava had 79% average recovery after cryopreservation via droplet vitrification (Dumet *et al*., 2013). Seed had no loss in viability after 14 y dry, hermetic storage at -20°C (RBG Kew, 2016)
Banana / plantain	*Musa*	**shoot tip**	[Except: *M. textilis*] *In vitro* shoot tips of 56 accessions (8 genomic groups of Musa spp. and one Ensete spp.) had 53% average post-thaw regeneration after droplet vitrification cryopreservation (Panis *et al*., 2005).
Rice	*Oryza*	seed	P50: *O. sativa* = 34 y (Walters *et al*., 2005). Seed viability constants: *O. sativa*, Ke 8.668; Cw 5.03; Ch0.0329; Cq 0.000478 (RBG Kew, 2016).
Pearl millet	*Pennisetum*	seed	Seed viability constants: *P. glaucum*, Ke 8.728; Cw 4.86; Ch0.0329; Cq 0.000478 (RBG Kew, 2016). At least another 19 species with orthodox seed storage behaviour (RBG Kew, 2016).
Beans	*Phaseolus*	seed	[Except: *P. polyanthus*]. P50: *P. vulgaris* = 31 y (Walters *et al*., 2005) Seed viability constants: *P. vulgaris*, Ke 9.09; Cw 4.761; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016). *P. lunatus* seed held under international standard seed bank conditions (RBG Kew, 2016). *P. maculatus* seed had 100 % viability after 15 % RH and 11 weeks at -20°C (RBG Kew, 2016). *P. microcarpus* seed had 100 % viability after 15 % RH and 46 days at -20°C (RBG Kew, 2016).
Pea	*Pisum*	seed	P50: *P. sativum* = 97 y (Walters *et al*., 2005) Seed viability constants: *P. sativum*, Ke 9.858; Cw 5.39; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016).
Rye	*Secale*	seed	P50: *S. cereale* = 36 y (Walters *et al*., 2005).
Potato	*Solanum*	**shoot tip**	[Except: *S. phureja*]. Full plant recovery after cryopreservation of 1028 accession of nine *Solanum* species / subspecies ranged from 34 to 59% for PVS2 treated *in vitro* shoot tips (Vollmer *et al*., 2016)
Eggplant	*Solanum*	seed	P50: *S. melongena* = 46 y (Walters *et al*., 2005)
Sorghum	*Sorghum*	seed	Seed viability constants: *S. bicolor*, Ke 10.588; Cw 6.305; Ch 0.041; Cq 0.000349 (RBG Kew, 2016).
Triticale	*Triticosecale*	seed	No problem for triticale seed storage under international standard seed bank conditions (RBG Kew, 2016).
Wheat	*Triticum*	seed	[Includes: *Agropyron* sp. (see Forage – grass below)]; *Elymus* sp. (P50s: *E. agropyroides* =19 y; *E. antarticus* =64 y; *E. batalinii* = 26y; *E. canadensis* = 25 y; *E. caninus* =18 y; *E. ciliaris* = 14 y; *E. dahuricus* = 22 y; *E. drobovii* = 16 y; *E. fibrosus* = 21 y; *E. hystrix* = 76 y; *E. lanceolatus* = 26 y; *E. mutabilis* = 20 y; *E. nutans* = 35 y; *E. patagonicus* = 73 y; *E. semicostatus* = 19 y; *E. sibericus* =80 y; *E. trachycaulus* = 14 y; *E. transhyrcanus* = 178 y; *E. tsukushiensis* = 38 y; *E. villosus* = 56 y; *E. virginicus* = 109 y (Walters *et al*., 2005); *Secale* sp. (P50: *S. cereale* = 36 y; Walters *et al*., 2005). Seed viability constants: *T. aestivum*, Ke 9.42 ; Cw 5.859 ; Ch 0.0329 Cq 0.000478 (RBG Kew, 2016)
Faba bean / vetch	*Vicia*	seed	P50: *Vicia* sp. = 71 y (Walters *et al*., 2005)
Cowpea *et al*.	*Vigna*	seed	P50: *V. radiata* = 457 y (Walters *et al*., 2005) Seed viability constants: *V. radiata*, Ke 10.858; Cw 6.27; Ch 0.0329; Cq 0.000478. *V. unguiculata* Ke 9.401; Cw 5.118; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016)
Maize	*Zea*	seed	[Excludes: *Z. perrenis, Z. diploperrenis, Z. luxurians*]. Seed viability constants: *Zea mays,* Ke 8.579; Cw 4.91; Ch 0.0329; Cq 0.000478 (RBG Kew, 2016)
Forage – legume			
Astragalus	3 species	seed	[Includes: *A. arenarius, A. chinensis*] *A. cicer* seed germination changed from 95 to 100% after 13 y storage under international standard seed bank conditions (RBG Kew, 2016).
Canavalia	*ensformis*	seed	[NB Six species in the genus are thought to be orthodox in storage response, and one species 'uncertain' (RBG Kew. 2016)]. Information needed on *C. ensiformis*.

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Crop	Genus	Material for storage	Example of preservation*
Coronilla	*varia*	seed	[NB Five in the genus are thought to be orthodox in storage response (RBG Kew. 2016)]. Information needed on *C. varia*.
Hedysarum	*coronarium*	seed	*H. coronarium* seed germination not change (98%) after 13 y under international standard seed bank conditions (RBG Kew, 2016).
Lathyrus	*odoratus*	seed	P50 = 40 y (Walters *et al*., 2005).
Lespedeza	*cuneata*	seed	P50 = 43 y (Walters *et al*., 2005).
Lotus	*cornicularis*	seed	P50 = 68 y (Walters *et al*., 2005).
Lupinus	*angustifolius*	seed	P50 = 41 y (Walters *et al*., 2005).
Medicago	6 species	seed	[Includes: *M. falcata*]. P50: *M. sativa* = 93 y (Walters *et al*., 2005). *M. arborea* seed had 100 % viability after 15 % RH and 4 weeks at -20°C (RBG Kew, 2016). *M. scutellata* seed had germination change from 99 to 95% after 13 y under international standard seed bank conditions (RBG Kew, 2016). *M. rigidula* seed had germination change from 97 to 99% after 13 y under international standard seed bank conditions (RBG Kew, 2016). *M. truncatula* seed germination remained 100% after 11 y under international standard seed bank conditions (RBG Kew, 2016).
Melilotus	*officinalis (*syn: *alba*)	seed	P50 = 63 y (Walters *et al*., 2005).
Onobrychis	*viciifolia*	seed	P50 = 34 y (Walters *et al*., 2005).
Ornithopus	*sativus*	seed	*O. sativus* seed germination not change (100%) after 13 y under international standard seed bank conditions (RBG Kew, 2016).
Prosopis	5 species	seed	[Includes: *P. affinis, P. alba, P. chilensis, P. nigra, P. pallida*] All these species are held in long-term storage (see RBG Kew, 2016).
Pueraria	*phaseoloides*	seed	*P. phaseoloides* average germination changed from 93 to 88% after 10 y under international standard seed bank conditions (RBG Kew, 2016).
Trifolium	15 species	seed	[Includes *T. agrocicerum*] 9/15 species with P50s: *T. alexandrinum* = 70 y; *T. ambiguum* = 86 y; *T. hybridum* = 29 y; *T. incarnatum* = 59 y; *T. pratense* = 43 y; *T. repens* = 77 y; *T. resupinatum* = 199 y; *T. subterraneum* = 73 y; *T. vesiculosum* = 46 y (Walters *et al*., 2005). *T. alpestre* seed germination changed from 89 to 98 % after 13 y under international standard seed bank conditions (RBG Kew, 2016). *T. angustifolium* seed germination of 97% after 12 y under international standard seed bank conditions (RBG Kew, 2016). *T. arvense* seed germination changed from 93 to 98% after 13 y under international standard seed bank conditions (RBG Kew, 2016). *T. rueppellianum* seed at 15 % RH tolerated 37 d at -20°C with 100% viability (RBG Kew, 2016). *T. semipilosum* seed at 15 % RH tolerated 1 month at -20°C with 100% viability (RBG Kew, 2016). Seed viability constants: *T. subterraneum*, Ke 7.21 ; Cw 3.51 ; Ch 0.04; Cq 0.0004 (RBG Kew, 2016)
Forage – grass			
Andropogon	*gayanus*	seed	*A. gayanus* seed is held in long-term storage (RBG Kew, 2016).
Agropyron	2 species	seed	P50s: *A. cristatum* = 65 y; *A. desertorum* = 45 y (Walters *et al*., 2005).
Agrostis	2 species	seed	[Includes: *A. tenuis*]. P50: *A. stolonifera* = 232 y (Walters *et al*., 2005).
Alopecurus	*pratensis*	seed	*A. pratensis* germination changed from 75 to 100% after 14 y under international standard seed bank conditions (RBG Kew, 2016).
Arrhenatherum	*eliatus*	seed	*A. eliatus* seed germination remained at 96% after 12 y under international standard seed bank conditions (RBG Kew, 2016).
Dactylis	*glomerata*	seed	P50 = 65 y (Walters *et al*., 2005).

Contd.

Crop	Genus	Material for storage	Example of preservation*
Festuca	6 species	seed	2/6 species with P50s: *F. arundinacea* = 25 y*; F. rubra* = 25 y (Walters *et al*., 2005). *F. giganea* seed germination changed from 94 to 93% after 12 y under international standard seed bank conditions (RBG Kew, 2016). *F. heterophylla* seed germination changed from 88 to 100% after 11 y under international standard seed bank conditions (RBG Kew, 2016). *F. ovina* seed germination changed from 95 to 96% after 12 y under international standard seed bank conditions (RBG Kew, 2016). *F pratensis* seed germination changed from 94 to 95% after 12 y under international standard seed bank conditions (RBG Kew, 2016).
Lolium	5 species	seed	[Includes: *L. hybridum*]. 4/5 species with P50s: *L. multiflorum* = 43 y; *L. perenne* = 41 y; *L. rigidum* = 82 y; *L. temulentum* = 263 y (Walters *et al*., 2005).
Phalaris	2 species	seed	*P. aquatic* seed germination remained c. 88% after 12 y under international standard seed bank conditions (RBG Kew, 2016). *P. arundinacea* seed held international standard seed bank conditions (RBG Kew, 2016).
Phleum	*pratense*	seed	P50 = 32 y (Walters *et al*., 2005). Seed viability constants: *P. pratense*, Ke 9.571; Cw 5.262; Ch 0.04; Cq 0.0004 (RBG Kew, 2016).
Poa	3 species	seed	P50: *P. pratensis* = 54 y (Walters *et al*., 2005). [Includes: *P. alpina; P. annua*]. *P. alpina* seed germination changed from 95 to 100% after 12 y under international standard seed bank conditions (RBG Kew, 2016). *P. annua* seed no problem for long-term storage under international standard seed bank conditions (RBG Kew, 2016).
Tripsacum	*laxum*	seed	Evidence needed.
Forage – other			
Atriplex	2 species	seed	Seeds of *A. halimus* are held in long-term storage (RBG Kew, 2016). Seeds of *A. nummularia* survive 14 days cryopreservation (Touchell and Dixon, 1993).
Salsola	*vermiculata*	seed	*S. vermiculata* seed maintained viability after 7 y storage at -15°C (Kay *et al*., 1988)
			Summary: 76 genera (NB *Lathyrus* and *Agropyron* are listed under both Food and Forage; *Solanum* is listed twice for potato and eggplant).

*Seed in the Walters *et al*. (2005) study was stored dry for >10 years at 5° C, plus c. 25 years at -18°C. P50 = estimated half-life. When P50 values were not available, information was drawn from the Seed Information Database, with information shown generally as average responses (RBG Kew, 2016).

apple, advantage is taken of adaptation to cope with cold winters as dormant buds can be cryopreserved, although very high levels of recovery growth are not yet guaranteed for all accessions and there is variability in response between years.

In summary, a range of techniques and protocols are available for the long-term *ex situ* preservation of the Annex I crops and crop complexes, but inter-specific and intra-specific variability in responses highlights the need to look beyond the one-size-fits-all approach to plant genetic resources *ex situ* preservation.

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