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Comparison of Seed Viability Among 42 Species Stored in a Genebank

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ABSTRACT This study was conducted to compare seed viability among 42 species after ten years of storage in the midterm storage complex (4℃, 30-40% RH) at the National Agrobiodiversity Center (NAC) Korean genebank maintained by the Rural Development Administration (RDA), Republic of Korea and to suggest the relative seed longevity and suitable monitoring intervals. The germination data from initial tests and after ten years of storage were compared to measure changes in viability during storage. The decline in seed viability varied greatly among seeds from -11.5% for *Triticum* sp. to 80% for melon. Coriander, crowndaisy, safflower, cosmos, Chinese-bellflower, waxgourd, melon, castorbean, Welch-onion, hollyhock, wild barley, and tallfescue showed significant decreases in viability of 34.2%, 73.4%, 36.5%, 30.0%, 40.2%, 71.3%, 80.0%, 65.9%, 45.5%, 51.4%, 53.0%, and 33.5%, respectively. Gardenpea, soybean, perilla, onion, wild rice, Italian-ryegrass, and pepper showed a 15-30% decline in viability, while the viability of morningglory, adzukibean, maize, and *Capsicum* sp. decreased by 15% to 5%. Chicory, radish, Chinese-cabbage, bottlegourd, watermelon, cucumber, pumpkin, *Cucurbita* sp., groundnut, kidneybean, clubwheat, sesame, wheat, *Triticum* sp., rice, barley, orchardgrass, buckwheat, and wild tomato showed changes in viability of <5%. The changes in storage viability also varied within families. The wild types of rice and barley showed rapid viability loss and presented different aspects from cultivars. Since seed viability of species, classified as index 1 or 2, showed germination losses >15% after ten years of storage, a viability test should be conducted with five year intervals, while species with germination loss of <15% (in index 3 or 4) can be retested at ten year intervals.

Keywords : seed longevity, seed quality, monitoring interval, genebank, active collection

Genebanks are storehouses of plant genetic resources that provide raw material for improvement of crops. Genebanks play a key role in the sustainable development of agriculture, helping to increase food production and thus overcome hunger and poverty. The seeds contained in genebanks are vital or irreplaceable resources that must be conserved to provide future agricultural options in a world facing climate change and other unforeseen challenges (Rao *et al*., 2006).

Seed genebanks maintain genetic resources within seeds over decades or centuries. Therefore, proper seed-handling procedures are fundamental to the long-term, cost-effective and efficient conservation of plant genetic resources. The goal of genebanks is to maintain high-viability accessions for long periods. However, mortality during storage or frequent regeneration poses the risk of genetic erosion (Parzies *et al*., 2000).

Seed viability testing is very important to ensure that the seeds being stored are capable of producing plants when sown in the field. Indeed, seeds must have high viability at the start of storage that is maintained throughout the storage period (Baskin & Baskin, 1988). Seed viability declines slowly at first, and then rapidly as seeds age (Roberts & Ellis, 1982); accordingly, it is important to know when this decline occurs so that the accession can be regenerated by replacing exiting seeds with high-viability ones. The International Standards for Genebanks (FAO/IPGRI, 1994) recommend that viability of seeds be determined before seeds are placed in the genebank and at regular intervals during storage. The first monitoring test should be conducted after ten years for seeds stored in base collections under preferred conditions (-18℃) with high initial viability (≥90% germination), while seeds stored in active collections under preferred conditions (4℃, 30-40% RH) should be monitored for viability after five years.

Thousands of seed viability tests have been performed

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after collection or regeneration of genetic resources and during conservation in genebanks. However, monitoring of all species in a genebank en bloc at five-or ten-year intervals is relatively time-consuming. Additionally, the aging rate characteristics and potential life spans of seeds vary among species (Hendry *et al*., 1994). Therefore, this study was conducted to predict seed longevity by comparing seed viability among 42 species held for ten years in midterm storage (4℃, 30-40% RH) and to provide guidelines for monitoring intervals of seed viability.

MATERIALS AND METHODS

Seed materials

In this study, the seeds of 42 species conserved in the mid-term storage complex maintained a 4℃ and 30-40% RH for ten years at the National Agrobiodiversity Center (NAC), the Rural Development Administration (RDA), Republic of Korea. The moisture content of seed materials placed into storage was controlled at 5-7% as per the regulations of the National Agrobiodiversity Center.

Seed viability test

A germination test was performed to determine the seed viability according to the standards of the International Seed Test Association (ISTA, 2005) or those of standard germination tests set by the Korean genebank (Table 1). Two replicates with 25-50 seeds per replicate were used to test germination. If seeds of species have bigger size than that of rice seeds, germination tests were carried out with 25 seeds per replicate and by 'Between paper (BP) method' using paper towel, otherwise with 50 seeds per replicate and by 'Top of paper (TP) method' using *petri dish* (Rao *et al*., 2006).

Data analysis

For seed lots that have been held for ten years at the Korean genebank, data for initial germination was extracted from the Germplasm Management System (National Agrobiodiversity Center Database). The germination data in the initial tests and after ten years of storage were compared to determine if there had been a significant drop in viability during storage. Species that showed a drop in germinability were identified by testing the null hypothesis of no difference between the two germination values. This was achieved by calculating the two-tailed probability using a t-test.

RESULTS AND DISCUSSION

Variation in seed viability between species

The viability of seeds of 42 species was tested after ten years of storage at 4℃ and 30-40% RH. There was wide variation across species in the level of decline in seed viability from -11.5% for *Triticum* sp. to 80% for melon (Table 2). Coriander, crowndaisy, safflower, cosmos, Chinese-bellflower, waxgourd, melon, castorbean, Welch-onion, hollyhock, wild barley, and tallfescue showed significant drops (≥ 30%) in viability of 34.2%, 73.4%, 36.5%, 30.0%, 40.2%, 71.3%, 80.0%, 65.9%, 45.5%, 51.4%, 53.0%, and 33.5%, respectively. Gardenpea, soybean, perilla, onion, wild rice, Italian-ryegrass, and pepper showed 15-30% declines in viability, while morningglory, adzukibean, maize, and *Capsicum* sp. showed decreases of 5-15%. The remaining species, chicory, radish, Chinese-cabbage, bottlegourd, watermelon, cucumber, pumpkin, *Cucurbita* sp., groundnut, kidneybean, clubwheat, sesame, wheat, *Triticum* sp., rice, barley, orchardgrass, buckwheat, and wild tomato showed changes of <5%.

We classified species into four categories (index 1 to 4) according to the intensity of the decline in viability (Table 3). Twelve species were included in index 1, which was classified as a decline of more than 30% in viability of seeds. Seven species were classified as index 2, which showed a decline 15-29%. Twenty-three species showed relatively stable storage as indicated by declines in viability of <15% and were categorized as index 3 to 4.

Some plant families had characteristically short-lived (Apiaceae and Brassicaceae) or long-lived (Malvaceae and Cucurbitaceae) seeds (Walters *et al.,* 2005). Rincker (1981) reported that seeds belonging to the *Festuca* genus had poor storage quality, while those belonging to the *Triticum* genus, tomato (*Solanum lycopersicum*) and corn (*Zea mays*) had relatively good storage quality. Similarly, in the present study, coriander (Apiaceae) and tallfescue (the *Festuca* genus) showed large decreases in viability. Five

Table 1. Specific germination conditions for 42 species used as experimental materials.

Family Name	Scientific Name	Common Name	Sub-strate	Temp. (°C)	Initial Count (Days)	Final Count (Days)	Treatment for dormancy breaking
Apiaceae	*Coriandrum sativum*	coriander	TP	20/30	7	21	-
Asteraceae	*Carthamus tinctorius*	safflower	BP	20/30	4	14	-
	Chrysanthemum coronarium var. coronarium	crowndaisy	TP	20/30	7	21	-
	Cichorium intybus	chicory	TP	20/30	5	14	-
	Cosmos bipinnatus	cosmos	TP	20/30	5	14	-
Brassicaceae	*Brassica rapa subsp. pekinensis*	Chinese -cabbage	TP	20/30	5	7	-
	Raphanussativus var. sativus	radish	TP	20/30	4	20	-
Campanulaceae	*Platycodon grandiflorum*	Chinese -bellflower	TP	20/30	7	14	-
Convolvulaceae	*Ipomoea nil*	morningglory	BP	20/30	7	14	-
Cucurbitaceae	*Benincasa hispida*	waxgourd	BP	20/30	4	14	-
	Citrullus lanatus var. lanatus	watermelon	BP	20/30	4	14	-
	Cucumis melo subsp. melo	melon	BP	20/30	4	8	-
	Cucumis sativus var. sativus	cucumber	BP	20/30	4	8	-
	Cucurbita moschata	pumpkin	BP	20/30	4	8	-
	Cucurbita sp.	-	BP	20/30	4	8	-
	Lagenaria siceraria	bottlegourd	BP	20/30	4	14	-
Euphorbiaceae	*Ricinus communis*	castorbean	BP	20/30	7	14	-
Fabaceae	*Arachis hypogaea*	groundnut	BP	20/30	5	10	-
	Glycine max	soybean	BP	25	5	8	-
	Phaseolus vulgaris var. vulgaris	kidneybean	BP	20/30	5	9	-
	Pisum sativum var. sativum	gardenpea	BP	20	4	8	scarification
	Vigna angularis var. angularis	adzukibean	BP	20/30	4	1	scarification
Lamiaceae	*Perilla frutescens var. frutescens*	perilla	TP	20	7	21	-
Liliaceae	*Allium cepa var. cepa*	onion	TP	20	6	12	-
	Allium fistulosum	Welsh-onion	TP	20	6	12	-
Malvaceae	*Alcea rosea*	hollyhock	TP	20/30	7	21	scarification
Pedaliaceae	*Sesamum indicum*	sesame	TP	20/30	3	6	-
Poaceae	*Dactylis glomerata*	orchardgrass	TP	20/30	7	21	-
	Festuca arundinacea	tallfescue	TP	20/30	7	14	-
	Hordeum bulbosum	wild barley	BP	20	4	7	-
	Hordeum vulgare subsp. vulgare	barley	BP	20	4	7	-
	Lolium multiflorum	Italian-ryegrass	TP	20/30	5	14	-
	Oryza nivara	wild rice	BP	25	7	14	-
	Oryza sativa	rice	BP	25	7	14	-
	Triticum aestivum subsp. aestivum	wheat	BP	20	4	8	-
	Triticum aestivum subsp. compactum	clubwheat	BP	20	4	8	-
	Triticum sp.	-	BP	20	4	8	-
	Zea mays subsp. mays	maize	BP	20/30	4	7	-
Polygonaceae	*Fagopyrum esculentum*	buckwheat	BP	20/30	4	7	-
Solaceae	*Capsicum annuum*	pepper	TP	20/30	7	14	-
	Capsicum sp.	-	TP	20/30	7	14	-
	Solanum pimpinellifolium	wild tomato	TP	20/30	5	14	-

Table 2. Comparison between family, genus and species displaying a drop in seed viability during ten years of storage at 4°C and 30-40% RH in the National Agrobiodiversity Center, Korea.

Family Name	Scientific Name	Common Name	No. of Accessions	Initial (A)	Final (B)	(A-B)±SE	Significance
Apiaceae	*Coriandrum sativum*	coriander	11	84.4	50.2	34.2±10.64	*z)
Asteraceae	*Carthamus tinctorius*	safflower	7	97.4	60.9	36.5±16.73	*
	Chrysanthemum coronarium var. coronarium	crowndaisy	10	99.0	25.6	73.4±5.86	*
	Cichorium intybus	chicory	4	81.5	79.3	2.2±14.44	
	Cosmos bipinnatus	cosmos	3	96.0	66.0	30.0±24.03	
Brassicaceae	*Brassica rapa subsp. pekinensis*	Chinese -cabbage	4	91.5	95.8	-4.3±6.61	
	Raphanus sativus var. sativus	radish	7	96.9	92.7	4.2±3.54	
Campanulaceae	*Platycodon grandiflorum*	Chinese -bellflower	8	96.8	56.6	40.2±13.08	*
Convolvulaceae	*Ipomoea nil*	morningglory	5	99.6	94.4	5.2±1.36	*
Cucurbitaceae	*Benincasa hispda*	waxgourd	3	92.0	20.7	71.3±8.67	*
	Citrullus lanatus var. lanatus	watermelon	46	90.6	89.4	1.2±2.39	
	Cucumis melo subsp. melo	melon	26	96.7	16.7	80.0±6.62	*
	Cucumis sativus var. sativus	cucumber	16	92.8	95.3	-2.5±4.94	
	Cucurbita moschata	pumpkin	26	88.7	87.5	1.2±3.57	
	Cucurbita sp.	-	15	91.7	96.7	-5.0±1.71	
	Lagenaria siceraria	bottlegourd	17	87.1	86.9	0.2±7.17	
Euphorbiaceae	*Ricinus communis*	castorbean	27	93.7	27.8	65.9±5.55	*
Fabaceae	*Arachis hypogaea*	groundnut	10	92.4	89.1	3.3±7.03	
	Glycine max	soybean	64	90.0	74.8	15.2±3.08	*
	Phaseolus vulgaris var. vulgaris	kidneybean	23	80.6	87.8	-7.2±5.12	
	Pisum sativum var. sativum	gardenpea	25	95.5	78.1	17.4±5.75	*
	Vigna angularis var. angularis	adzukibean	28	86.1	79.0	7.1±4.07	*
Lamiaceae	*Perilla frutescens var. frutescens*	perilla	23	91.5	72.9	18.6±4.24	*z)
Liliaceae	*Allium cepa var. cepa*	onion	78	95.5	66.6	28.9±2.31	*
	Allium fistulosum	Welsh-onion	6	96.0	50.5	45.5±11.30	*
Malvaceae	*Alcea rosea*	hollyhock	3	87.7	36.3	51.4±19.12	
Pedaliaceae	*Sesamum indicum*	sesame	71	91.3	90.7	0.6±1.63	
Poaceae	*Dactylis glomerata*	orchardgrass	30	90.1	88.6	1.5±3.28	
	Festuca arundinacea	tallfescue	11	96.8	51.4	45.4±4.83	*
	Hordeum bulbosum	wild barley	6	99.7	46.7	53.0±14.54	*
	Hordeum vulgare subsp. vulgare	barley	44	87.0	93.9	-6.9±2.84	
	Lolium multiflorum	Italian-ryegrass	20	96.0	71.1	24.9±1.77	*
	Oryza nivara	wild rice	5	97.2	81.4	15.8±9.52	
	Oryza sativa	rice	12	95.0	90.1	4.9±2.00	*
	Triticum aestivum subsp. aestivum	wheat	329	91.8	86.9	4.9±0.94	*
	Triticum aestivum subsp. compactum	clubwheat	12	90.5	94.3	-3.8±3.51	
	Triticum sp.	-	298	86.2	97.7	-11.5±0.94	
	Zea mays subsp. mays	maize	6	98.0	89.7	8.3±3.84	*
Polygonaceae	*Fagopyrum esculentum*	buckwheat	10	98.4	98.9	-0.5±0.5	
Solaceae	*Capsicum annuum*	pepper	96	94.2	78.7	15.5±2.02	*
	Capsicum sp.	-	33	94.5	82.2	12.3±3.04	*
	Solanum pimpinellifolium	wild tomato	11	91.3	91.8	-0.5±4.19	

*z) : significant ($p<0.05$)

species (watermelon, cucumber, pumpkin, *Cucurbita* sp., and bottlegourd) in Cucurbitaceae, three species (wheat, clubwheat, *Triticum* sp.) in *Triticum*, and wide tomato and maize maintained high viability. In contrast, hollyhock in Malvaceae showed 51.4% viability loss, indicating that it was short lived, while radish and Chinese-cabbage in Brassicaceae showed nearly constant seed viability after ten years of storage.

Seeds of some species are genetically and chemically equipped for longer storability than others under similar conditions. For example, seeds of *Canna* (Sivori *et al.*, 1968), *Lotus* (Wester, 1973), and *Lupinus* (Porsild & Harrington, 1967) have been reported to be viable even after 500 years. Other hard seeded genera reported by Harrington (1972) to be germinable after 100 years include *Albizia*, *Cassia*, and *Trifolium*. However, seeds of other species such as orchardgrass, soybean, lettuce, onion and rye are characteristically short-lived (Copeland & McDonald, 2001). In this study, onion and Welsh-onion (genus *Allium* in family Liliaceae) showed reductions in viability of 28.9% and 45.5%, respectively. However, orchardgrass showed only a small decrease in viability of 1.5%.

The change in storage viability also varied within families. For example, in Asterceae, crown daisy, safflower, and cosmos were classified as index 1, but chicory as index 4. Similarly, in Cucurbitaceae, the seed viability of waxgourd and melon decreased rapidly, whereas that of bottlegourd, watermelon, cucumber, pumpkin, and *Cucurbita* sp. did not. In Fabaceae, gardenpea and soybean showed an intermediate decline in seed viability, but adzukibean, groundnut, and kidneybean showed only slight and insignificant changes in seed viability. In Poaceae, wild rice, wild barley, Italian-ryegrass, and tallfescue showed large decreases in viability, while others (clubwheat, wheat, *Triticum* sp., rice, barley, and orchardgrass) did not. Walters *et al.* (2005) reported that large families such as Asteraceae, Fabaceae, Poaceae, and Solanaceae contained species with wide-ranging P_{50} values, that is the time for germination to decrease to half the initial value using the coefficients of Avrami viability equations (Avrami, 1941). Similarly, Probert *et al.* (2009) reported wide variation in P_{50} between families, as well as within some families, but relatively little variation in longevity among genera within those families.

It is interesting that the wild types of rice and barley showed rapid viability loss and presented different aspects with cultivars. Niedzielski *et al.* (2009) reported that wild rye (*Secale strictum*) grains aged somewhat more rapidly than cultivated rye (*S. cereale*) grains under genebank conditions. Differences in seed longevity between cultivars and wild types of crops have been reported for diverse taxa (Walters *et al.*, 2005; Ellis & Hong, 2007), although there appears to be no consistent trends.

Many studies have suggested that oilseeds are short-lived (Nagel & Börner, 2010); however, the results of the present investigation are not consistent with these results (data not shown). Walters *et al*. (2005) suggested that anecdotal accounts of poor storage quality of lettuce or peanut seeds have led to the widespread idea that seeds with high oil content store poorly. Indeed, the purported link between high oil content and short storage life-span has not been supported by recent analyses. Probert *et al.* (2009) reported that seed oil content was not correlated with P_{50}, but that seed longevity was related to seed structure and climate of origin. Therefore, this survey showed that seed deterioration is not related to seed oil contents.

Implications for monitoring of seed viability in genebank

Table 3 presents the ranking of 42 species according to the level of viability loss after testing germination. Understanding differences in seed longevity among species is crucial to the effective management of seed conservation and collections because it influences the selection of viability retest intervals and hence regeneration or re-collection strategies (Probert *et al.*, 2009).

Seed longevity has long been thought of as a quantitative trait that is controlled in minor ways by numerous genes scattered throughout the genome and is influenced by environmental conditions (Kochanek *et al.*, 2010). The environmental factors influencing longevity include parental (pre-zygotic and post-zygotic) environment (Daws *et al.*, 2004), timing of harvest (Wang *et al*., 2008), postharvest processing (Hay *et al.*, 2006), and storage environment, especially, temperature and relative humidity (Roberts & Ellis, 1989; Walters, 1998). Seed structure and climate of origin are also known genetic factors related to seed

Table 3. Monitoring period predicted seed longevity by testing germination of 42 species based on 1,489 accession seeds stored for ten years at 4°C and 30-40% RT.

Index	1	2	3	4
Decreasing rate of seed viability	>30%	15-30%	5-15%	<5%
Seed longevity	(Very short)	(Short)	(Medium)	(Long)
Crop names	coriander safflower crowndaisy cosmos Chinese-bellflower waxgourd melon castorbean Welsh-onion hollyhock tallfescue wild barley	soybean gardenpea perilla onion Italian-ryegrass wild rice pepper	morningglory adzukibean maize *Capsicum* sp.	chicory Chinese-cabbage radish watermelon cucumber pumpkin *Cucurbita* sp. bottlegourd groundnut kidneybean sesame orchardgrass barley rice wheat clubwheat *Triticum* sp. buckwheat wild tomato

longevity (Probert *et al*., 2009). Furthermore, Miura *et al.* (2002) detected three quantitative trait loci (QTLs) for seed longevity in rice. Genetic differences in storage potential are not limited to seeds of different species, and differences in seed storability may occur among cultivars (Kueneman, 1983; Niedzielski *et al.*, 2009) or between genotypes of a species (Nagel *et al*., 2010).

Current guidelines (Rao *et al*., 2006) recommend that base and active collections stored according to international standards (FAO/IPGRI, 1994) be tested for viability and to determine whether the seeds have poor longevity. The FAO/IPGRI (1994) recommends that the initial germination value exceed 85% for most seeds and that regeneration should be undertaken when viability falls below 85% of the initial value. However, frequent germination tests are expensive and time-consuming and can also be a cause of gene modification. Additionally, the monitoring interval depends on species, storage environment, seed viability at the beginning of storage, and capacity of the genebank. The interval between subsequent tests should be based on experience, and may be adjusted up or down depending on the extent of viability loss observed during the first monitoring test. Although species included in index 1 or 2 should be retested at intervals of 5 years interval according to the recommendations of the ISTA, species in index 3 or 4 can be checked with a ten year interval.

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