Effects of Seed Size and Several Factors on Ultra-drying and Germination of Ultra-dried Seeds in Soybean

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ABSTRACT : Ultra-drying [<5.0% seed moisture content (SMC)] storage technique is a cost-effective storage method for oily seeds. To decide proper ultra-drying condition for soybean seeds, drying rate was compared three silica gel to seed ratios, two seed sizes with varietal difference, two kinds of container, and three seed amounts per container under 23 ± 1°C. When the relative humidity (RH) was reduced at the rate of less than 0.1% a day, silica gel was replaced with dry one by 47 days. Higher silica gel to seed ratios (3 : 1 and 2 : 1) dried faster than lower ratio (1 : 1) until 28 days, but not after 43 days of drying. Also, large seeded variety was dried faster than small seeded variety. Kinds of container and seed amounts per container didn't show differences in drying of soybean seeds. After completion of ultra-drying, percentage germination by standard germination test (SGT) was not different among silica gel to seed ratios, kinds of container, and seed amounts per container, except among seed sizes (varieties). Before SGT, soybean seeds were premoistened using saturated $CaCl_2$ for 48 hours and NH_4Cl for 24 hours in desiccators. To compare germinability between ordinary-dried seeds and ultra-dried seeds, the seeds of seven soybean varieties, which were varying in size from 8.1 to 34.9 g per 100 seeds, were dried using same amount of silica gel under 23 ± 1°C. After completion of 76 days of drying, SMCs were reduced to 3.13~3.45% from 7.86~8.82%. SMC after completion of drying was not correlated with 100-seed weight (r=0.556). Before germination tests, soybean seeds were premoistened using saturated salt solutions. Percentage germination was higher with ultra-dried seeds than ordinary-dried seeds in SGT and higher with ordinary-dried seeds than ultra-dried seeds in AAT at the beginning of storage and after 6 months storage, but general trend of percentage germination was not observed among varieties classified by 100-seed weight. From these results, we concluded that further studies are needed to improve ultra-drying storage method for soybean seeds.

Keywords **:** soybean, ultra-drying, seed size, seed moisture content, standard germination test, accelerated aging test, percentage germination, silica gel.

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Storage life of seeds mainly depends on seed moisture content (SMC) and storage temperature. Soybean germplasm for long-term storage are usually stored in hermetically sealed aluminum foil bags under -10~-20°C and 8% SMC, those for mid-term storage are stored under 5~15°C and 45% relative humidity (RH), and those for a year of storage are stored under ambient temperature condition (Juvik *et al.*, 1985). Harrington (1973) proposed that each 1% reduction in SMC doubles the life of seeds and each 5°C reduction in seed storage temperature doubles the life of the seeds. Ellis *et al.*(1986, 1989) reported that longevity of seeds of several crops could be greatly increased by storage under ultra-dry state (<5% SMC). The increase in longevity of sesame seeds which results from reducing SMC from 5 to 2% is about the same as that achieved by reducing storage temperature from +20 to -20°C (Ellis *et al.*, 1986). Therefore such ultra-dry may be low cost alternative to the use of sub-zero temperature for long-term seed storage.

The lower MC limits in hermetic storage, which did not influence longevity were 2 to 6% depending on crops (Roberts & Ellis, 1969). Also, Ellis *et al.* (1990) reported that lower SMC limit, which did not influence longevity was 3.3% (11.4%, RH) in soybean. Cheng *et al.* (1990) reported that ultra-drying treatment did not induce any significant changes in seed germination and vigour in Chinese cabbage and cotyledon cells after ultra-drying maintained their integrity even after imbibition without prehumidification. The ability of soybean seeds to withstand dehydration indicates that their membranes may maintain structural integrity even when dry.

Rehydration process is generally known to cause more injury than ultra-drying process. Hobbs & Obendorf (1972) reported that 13 to 14% SMC (dry weight basis) was the lowest SMC resulting in optimum protection against chilling temperatures during imbibition in both 'Chippewa 64' and 'Acme' soybean varieties. With SMC of 5%, exposure to chilling during imbibition or seed soaking resulted in decreased survival and seedling vigour and the combination of anaerobic soaking at 5°C was nearly fatal. However, losses were avoided by equilibrating seeds to 13% SMC before imbibition (Hobbs & Obendorf, 1972). Cseresnyes &

Vorovenci (1984) reported that the rapid water absorption rate was greater for cracked seeds and seeds with low SMC, and low temperature during water absorption also negatively affected seed germination. Schultz (1979) suggested the preconditioning of mechanically damaged seeds and of seeds with low moisture content, at 30°C in a vapour saturated atmosphere.

To reduce SMC, the use of a dehumidifier or silica gel for seed drying at lower temperature in genebanks has been recommended (Hansen, 1985). Silica gel drying is particularly suitable for genebanks which have small germplasm collections. But there is only limited information available concerning the use of silica gel for drying of soybean seeds.

This study investigated the effects of silica gel to seed ratios, seed sizes, kinds of container for drying, and seed amounts per container for development of ultra-drying method. Also standard germination test (SGT) and accelerated ageing test (AAT) were done for ordinary-dried seeds and ultra-dried seeds at the beginning of storage and 6 months after storage under 25°C condition for comparison of varietal effects of seed size and SMC on the germination of soybean seeds.

MATERIALS AND METHODS

Determination of proper ultra-drying conditions for soybean seeds using silica gel

This experiment was conducted at the Asian Vegetable Research and Development Center (AVRDC), Taiwan in 1994. The seeds of two soybean varieties different in size, SS86013-7-4 with 26.5 g per 100 seeds and IAC 100 with 11.5 g per 100 seeds, were used for this experiment. The seeds used, were stored under short-term storage condition at the Genetic Resources and Seed Unit (GRSU), AVRDC. To make ultra-drying state, soybean seeds were put into two kinds of container (iron screen and cheese cloth) and placed on the upper part of desiccators (30 cm in diameter) and silica gel was placed on the bottom of desiccators under 23 ± 1°C. Seed amounts per container were 25, 50, and 100 g each. All factors have 3 replications. When the RH, which was monitored every day using Barigo's precision-hygrometer-superatherm (0~100% RH) for 47 days, was decreased at the rate less than 0.1% a day, silica gel was replaced by the same amount of dry silica gel (round shape). Seed weight was checked every time when silica gel was replaced. Before SGT, ultra-dried seeds were placed upper part in the desiccators (30 cm in diameter) containing saturated $CaCl_2$ (aq) on the bottom for 48 hours, then seeds were shifted into the desiccators containing saturated NH_4Cl (aq) on the bottom for 24 hours. The premoistening treatment using saturated solutions was done under 20°C. SGT was conducted according to the ISTA rule (1985) using 3 replicates of 50 seeds each. Rolled towel test was used with 8 hours for light condition at 30°C and 16 hours for dark condition at 20°C.

SMC was calculated by the formula "SMC (%) = [original SMC (%) – reduced seed weight (%)] / [100 – reduced seed weight (%)]".

All collected data were subjected to analysis of variance and correlation using Statistical Analysis System (SAS, ver. 6.02) with pc package.

Varietal effects of seed size on the ultra-drying and germination of ordinary-dried and ultra-dried soybean seeds

This experiment was conducted at the AVRDC, Taiwan in 1994 to 1995. The seeds of seven soybean varieties varying in size from 8.1 to 34.9 g per 100 seeds, had been stored under short-term storage condition at the GRSU, AVRDC and were dried using same amount of silica gel in the desiccators (30 cm in diameter) under 23 ± 1°C. After completion of 76 days of ultra-drying, ordinary-dried seeds and ultra-dried seeds were sealed with aluminum foil bags and stored for 6 months under 25°C, and determined in percentage germination by SGT and AAT. Accelerated aging was conducted under 100% RH and 41°C for 72 hours in the water bath (Precision Scientific model 270). Germination tests (including SGT and AAT) were conducted using 4 replicates of 50 seeds each.

The methods for monitoring of RH, premoistening of dried seeds, germination tests, and statistical analysis were the same as the methods used in determination of proper ultra-drying conditions using silica gel.

RESULTS AND DISCUSSION

Determination of proper ultra-drying conditions for soybean seeds using silica gel

Changes in SMC of soybean according to different treatments, silica gel to seed ratios, varieties, containers, and seed amounts per container are shown in Table 1. SMC of soybean was reduced to 4.0~4.2% from 8.7% after 47 days of drying. Higher silica gel to seed ratios (3 : 1 and 2 : 1) dried seeds faster than 1 : 1 silica gel to seed ratio until 28 days of drying. Also, large seeded SS86013-7-4 (26.5 g/100 seeds) was dried faster than small seeded IAC 100 (11.5 g/ 100 seeds). Kinds of container and seed amounts per container didn't show differences in drying of soybean seeds

Table 1. Changes in seed moisture content of soybean by silica gel to seed ratios, varieties, containers, and seed amounts per container during drying by silica gel in desiccators under 23 ± 1°C.

Silica gel to seed ratio	Variety†	Container	13 days‡			28 days			43 days			47 days
			25 g§	50 g	100 g	25 g	50 g	100 g	25 g	50 g	100 g	100 g
1 : 1	SS 86013-7-4	IS¶	6.1††	6.1	6.1	4.6	4.6	4.6	4.1	4.1	4.1	4.0
		CC#	5.9	6.1	6.2	4.6	4.6	4.5	4.2	4.3	4.1	4.1
	IAC 100	IS	6.4	6.4	6.3	4.8	4.7	4.7	4.4	4.3	4.2	4.1
		CC	6.5	6.4	6.2	4.9	4.7	4.6	4.4	4.3	4.2	4.1
2 : 1	SS 86013-7-4	IS	5.9	5.8	5.9	4.4	4.6	4.6	4.2	4.2	4.2	4.1
		CC	5.7	5.9	5.9	4.6	4.5	4.6	4.3	4.2	4.2	4.1
	IAC 100	IS	6.0	6.0	6.1	4.4	4.6	4.7	4.3	4.2	4.3	4.2
		CC	5.9	6.1	6.0	4.3	4.6	4.6	4.1	4.1	4.2	4.2
3 : 1	SS 86013-7-4	IS	5.6	5.9	5.7	4.6	4.6	4.5	4.2	4.2	4.1	4.0
		CC	5.5	5.7	5.8	4.3	4.6	4.6	4.1	4.3	4.2	4.2
	IAC 100	IS	5.7	5.7	5.9	4.5	4.7	4.6	4.1	4.2	4.2	4.2
		CC	6.1	5.9	5.8	4.7	4.7	4.7	4.3	4.4	4.2	4.1

†Original moisture contents were 8.76% in SS86013-7-4 and 8.74% in IAC 100.
‡Drying period. §Seed amount per container.
¶IS: iron screen. #CC: cheese cloth.
††Moisture content (MC) was calculated by MC (%) = [original MC (%) − reduced seed weight (%)] ÷ [100 − reduced seed weight (%)]

Table 2. Combined analysis of seed moisture contents of soybean by silica gel to seed ratios, varieties, containers, and seed amounts per container during drying by silica gel in desiccator under 23 ± 1°C.

Source of variance	DF	MS by drying duration		
		13 days	28 days	43 days
Silica gel to seed ratio (a)	2	0.6502**	0.0315*	0.0008^{ns}
Variety (b)	1	0.3211**	0.1122**	0.0514**
Container (c)	1	0.0000^{ns}	0.0006^{ns}	0.0040^{ns}
Seed amount per container (d)	2	0.0137^{ns}	0.0142^{ns}	0.0066^{ns}
a × b	2	0.0111^{ns}	0.0322^{ns}	0.0148^{ns}
a × c	2	0.0032^{ns}	0.0032^{ns}	0.0067^{ns}
a × d	4	0.0030^{ns}	0.0377^{ns}	0.0151^{ns}
b × c	1	0.0278^{ns}	0.0008^{ns}	0.0028^{ns}
b × d	2	0.0325^{ns}	0.0003^{ns}	0.0048^{ns}
c × d	2	0.2027^{ns}	0.0003^{ns}	0.0023^{ns}
Error	16	0.0140	0.0087	0.0054

*,**Significant at the 0.05 and 0.01 probability levels, respectively.
^{ns}Not significant at the 0.05 probability level.

(Table 2). Zhang & Tao (1988) reported that silica gel was capable of lowering SMC to 3~7% within a month. It took more time in our study than theirs. We suppose that the difference in the drying rate between two studies be caused by differences in species, seed size, initial SMC, and changing interval of silica gel. Zhang & Tao (1988) reported that the ratio of silica gel to seed significantly affected the rate of seed drying, particularly for seeds with high initial SMCs and large seed size. Our study was also in good agreement with their study using high silica gel to seed ratios at the relatively high SMCs.

Table 3. Germinability of ultra-dried soybean seeds according to silica gel to seed ratios, varieties, containers, and seed amounts per container by standard germination test.

Silica gel to seed ratio	Variety	Container	Germination (%)
1 : 1	SS 86013-7-4	IS†	98.7
		CC‡	95.3
	IAC 100	IS	94.0
		CC	96.3
2 : 1	SS 86013-7-4	IS	97.3
		CC	96.7
	IAC 100	IS	95.7
		CC	94.7
3 : 1	SS 86013-7-4	IS	98.7
		CC	98.0
	IAC 100	IS	96.3
		CC	95.6
	SS 86013-7-4		97.5a§
	IAC 100		95.4b

†IS : iron screon, ‡CC : cheese cloth.
§Within columns, means followed by the different letters are significantly different according to LSD (0.01).

SGT was conducted to determine germinability of ultra-dried seeds. The result of SGT showed 94% or more in percentage germination and percentage germinations did not differ among silica gel to seed ratios, between kinds of container, and among seed amounts per container. There was, however, significant difference in percentage germination between varieties. The large seeded SS86013-7-4 showed 97.5% germination and small seeded IAC 100 showed 95.4% germination (Table 3). Zhang & Tao (1988) reported that 3:1 silica gel to seed ratio decreased seed germination. The results of our study were different from their result. This result means that ultra-drying is not detrimental to soybean seeds. We suppose that the difference in the detrimental effect of ultra-dried seeds on seed germination between two studies be caused by differences in species.

Varietal effects of seed size on the ultra-drying and germination of ordinary-dried and ultra-dried soybean seeds

Varietal effects of seed sizes (7 varieties varying in 100-seed weight from 8.1 g to 34.9 g) on the SMC of soybean after 76 days of drying using silica gel in desiccators under 23 ± 1°C are shown in Table 4. SMC of soybean were reduced to 3.13~3.45% from 7.86~8.82% after 76 days of drying.

Table 4. Varietal effects of seed sizes on the seed moisture contents of soybean after 76 days drying by silica gel† in desiccator under 23 ± 1°C.

Variety	100-seed weight (g)	Original seed moisture content (%)	Reduced seed weight (%)	Dried seed moisture content (%)
IAC 80-4228	8.1	8.05	4.95c‡	3.26bc‡
GC84056-1-2-2-1	9.9	7.86	4.88c	3.13c
AGS 181	15.9	8.53	5.26b	3.45a
GC87017-40-B-2	20.7	7.98	4.90c	3.24bc
SS86013-7-4	26.5	8.20	4.99c	3.38ab
G 9053	31.2	8.03	4.98c	3.21c
AGS 292	34.9	8.82	5.73a	3.28bc
Mean	21.0	8.21	5.10	3.28

†Silica gel to seed ratio was 1 : 1.
‡Within columns, numbers followed by the same letter are not significantly different according to Duncan's multiple range test at 0.05 probability level.

The percentages of reduced seed weight by drying were largest in AGS 292 (34.9 g per 100 seeds) and smallest in GC84056-1-2-2-1 (9.9 g per 100 seeds). But percentage of reduced seed weight was not correlated with 100-seed weight (r = 0.159) and original SMC (r = 0.334). After completion of 76 days of drying, AGS 181 (15.9 g per 100 seeds) showed the highest and GC84056-1-2-2-1 (9.9 g per 100 seeds) showed the lowest in SMC. SMC after drying was highly correlated with original SMC (r = 0.961), but not correlated with 100-seed weight (r = .556). Zhang & Tao (1988) insisted that seed size is an important factor affecting seed drying. But we did not find any relationship between seed size and seed drying rate in this study.

Changes in percentage germination of soybean seeds as affecter by types of germination test and drying conditions at the beginning of storage and after 6 months storage under 25°C are shown in Table 5. In SGT, mean percentage germinations were over 95% at the beginning of storage, then reduced to 84.7% for ordinary-dried seeds and 88.5% for ultra-dried seeds after 6 months storage. In AAT, mean percentage germinations were 91.1% for ordinary-dried seeds and 82.4% for ultra-dried seeds at the beginning of storage, and 62.4% for ordinary-dried seeds and 28.1% for ultra-dried seeds after 6 months storage.

Among varieties, percentage germinations were highly significantly different at the beginning of storage and after 6 months storage (Table 6). GC84056-1-2-2-1, SS86013-7-4, and AGS 292 showed over 90% germination for both ordinary-dried seeds and ultra-dried seeds in SGT after the 6 months storage. Also, GC84056-1-2-2-1, AGS 181, and AGS 292 showed over 70% germination for ordinary-dried seeds and over 40% germination for ultra-dried seeds in AAT after the 6 months storage (Table 5). Therefore, we suggest that GC84056-1-2-2-1 and AGS 292 , which were

Table 5. Genotypic changes in percentage germination of soybean seeds as affected by types of germination test, and drying conditions at the beginning of storage and after 6 months storage under 25°C.

Variety	100-seed weight (g)	Beginning of storage				After 6 months storage			
		SGT†		AAT‡		SGT		AAT	
		OD§	UD¶	OD	UD	OD	UD	OD	UD
IAC 80-4228	8.1	91.5	98.5	94.0	84.5	76.5	95.5	28.0	15.5
GC84056-1-2-2-1	9.9	99.0	99.5	96.5	95.5	97.5	96.5	74.5	43.5
AGS 181	15.9	90.0	97.0	92.0	87.5	78.5	94.5	73.5	41.5
GC87017-40-B-2	20.7	97.5	100.0	90.0	94.0	84.5	94.5	77.5	25.5
SS86013-7-4	26.5	98.5	99.5	92.0	90.5	95.0	94.5	65.5	26.5
G 9053	31.2	91.5	86.5	77.5	42.5	65.0	52.0	36.0	4.5
AGS 292	34.9	97.5	96.5	95.5	82.5	96.0	92.0	82.0	40.5
Mean	21.0	95.1	96.8	91.1	82.4	84.7	88.5	62.4	28.1

†SGT: standard germination test. ‡AAT: accelerated ageing test.
§OD: ordinary drying, 7.8~8.8% in seed moisture content.
¶UD: ultra-drying, 3.1~3.5% in seed moisture content.

Table 6. Combined analysis of percentages germination of soybean seeds by varieties, types of germination test, and drying conditions at the beginning of storage and after 6 months storage under 25°C.

Source of variance	DF	MS by storage duration	
		0 month	6 months
Variety (a)	6	936.20**	2,832.88**
Drying condition (b)	1	393.75**	4,413.44**
Type of germination test (c)	1	2,143.75**	48,626.33**
a × b	6	246.25**	388.95*
a × c	6	347.92**	477.81**
b × c	1	670.32**	8,739.01**
a × b × c	6	132.15**	366.07*

*,**Significant at the 0.05 and 0.01 probability levels, respectively.

bred for the tropical Asia at the AVRDC, have high storage ability. Between drying condition, percentage germination was highly significantly different at the beginning of storage and after 6 months storage (Table 6). Ultra-dried seeds showed higher germination rate than ordinary-dried seeds under SGT, but ordinary-dried seeds did higher germination rate than ultra-dried seeds under AAT (Table 5). There were highly significant differences in percentage germinations between types of germination test at beginning of storage and after 6 months storage (Table 6). Also, significant interactions were found among varieties × drying conditions, varieties × types of germination test, drying conditions × types of germination test, and varieties × drying conditions × types of germination test at the beginning of storage and after 6 months storage (Table 6).

Shen & Qi (1998) suggested that drying to extremely low water contents has detrimental effects on longevity. The SMC of ultra-dried soybean seeds used in this study was consistent with the results by Cheng *et al.* (1990) that lower SMC limit did not influence longevity of Chinese cabbage seeds. However, lower germinability of ultra-dried seeds in AAT means that further studies are needed to improve ultra-drying storage method using for soybean seeds using silica gel.

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