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Differences of Water Absorption Property and Seed Viability according to Morphological Characters in Soybean Genotypes

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ABSTRACT : The impermeable seed coat is valuable trait in soybean because impermeable seed retain viability for longer period than permeable seed under adverse conditions such as delayed harvest or prolonged storage. Soybean seeds of various size showing different seed hardness were examined for their water absorption and seed viability under adverse storage conditions. Of one hundred thirty nine genotypes, eight types of seeds having different seed hardness and seed size were used as material. Soybean genotypes showing high hard seed rate, GSI13125 (89%), GSI10715 (54%), and GSI10284 (42%), were slow in water absorption and low in the electroconductivity of seed leachate in distilled water. Germination of GSI10284 and GSI13125 that have higher hard seed rate was less affected by CSVT and artificial aging treatment indicating higher seed storability. The higher storing ability of both collections was confirmed by electroconductivity test for leachate. GSI10122 showed low seedling emergence when the seeds were artificially aged. This genotype was considered as to having a poor storing ability based on difference of electroconductivity before and after artificial aging. Among tests conducted in the experiment, CSVT could be used for determining storage life in legumes. In conclusion, water absorption property of seed was strongly related to the hardness that is directly related to the seed viability and storing ability in soybean seed.

Keywords: soybean, water absorption, seed viability, hard seed, complex stressing vigour test

Impermeability of seed by seed coat is closely related to the storability of seed. Water absorption property is affected by anatomical feature of palisade cell layer of seed coat. Because seed coat controls water imbibition and gas exchange of seed and impermeable seed coat has a small number of pores and/or high-waxy layer, the seed having impermeable seed coat shows low exchange rate of gas and water and higher seed viability for prolonged period (Hartwig & Potts, 1987; McDonald *et al.*, 1988; Ragus, 1987; Yaklich *et al.*, 1986). Therefore, the seed showing high impermeability have higher seed viability than that showing low impermeability under adverse storage condition or delayed harvest (Hartwig & Potts, 1987; Yaklich *et al.*, 1986).

Seed coat consisted of palisade cell layer, hourglass cell layer, and parenchyma cell layer connected to hypocotyls. The seed coat of hard seed have thick palisade layer and thin hourglass cell layer. There is a close relationship between seed color and seed coat thickness. Palisade cell layer of black soybean is thicker than that of yellow soybean (Atechavaleta & Synder, 1981; Park *et al.*, 1998). Pores on seed coat formed at late maturity in soybean are also related to the water permeability (Ragus, 1987). Yaklich *et al.* (1986) reported that seed size and pore density influence to the water absorption in soybean. In addition, water absorption of soybean is affected by surface area, soaking temperature, salt concentration and composition of soaking solution (Ragus, 1987; Yaklich *et al.*, 1986). Many researchers reported that water absorption is dependent on seed size in soybean. In general, small seed shows higher water permeability than large seed (Park *et al.*, 1996), most hard seeds are small in seed size and slow in water uptake and germination under normal condition (Ragus, 1987). Seed color controlled by seed coat color is related to the hardness of seed. Park *et al.* (1998) reported that yellow soybean showed extremely low (0%) hard seed rate, on the other hand, two black soybean cultivars, Geumjeongkong #1 and #2, showed 1.7% and 7.3%, respectively, in hard seed rate. Park *et al.* (1996) suggested moisture uptake is dependent on the color of soybean seed. In their results, black seeds showed slower water absorption rate and lower electrocondiuctivity of leachate as compared with yellow seeds. The low rate of water absorption may be due to the deposition of wax on seed coat (Park *et al.*,1996). In the other experiment, small soybean seed with black or yellow seed coat showed lower electroconductivity of leachate from the artificially aged seeds than large seed with brown or green seed coat in 50 soybean land races (Kwon *et al.*, 1984).

In this experiment, we have tried to verify the appropriate method for knowing the seed viability and to clarify the water absorption property of soybean that shows persisting seed viability under long storage condition to select proper soybean germplasm for improving storability.

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MATERIALS AND METHODS

Seed samples

One hundred thirty nine genotypes screened by field germination test from 2,105 genotypes stored without controlling temperature and humidity. Those germinated genotypes were proliferated and used as material. Of 139 selected genotyes, eight genotype, GSI10284, GSI10122, GSI13815, GSI10715, GSI13125, GSI13833, GSI13866 and Taekwangkong (recommended variety), were selected and used for subsequent experiment and seed hardness and morphological characteristics of selected seeds were shown at Fig. 1 and Table 1.

Control of seed moisture equilibrium

Seed moisture was equilibrated by placing seeds on wire mesh screen tray placed in plastic air-tight box containing glycerol solution at 25 °C for 72 hr. Relative humidity was controlled to 70% by adjusting the concentration of glycerol solution. The moisture content of moisture-equilibrated seeds was measured after drying at 103 °C for 17 hr. In this experiment, moisture content of moisture-equilibrated seeds was 7.8 %.

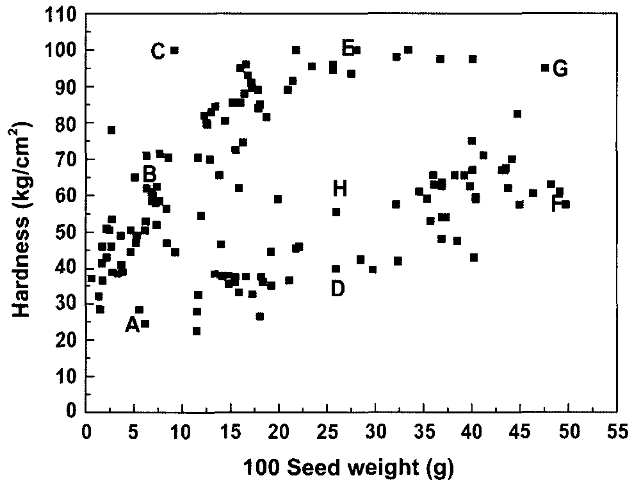

Fig. 1. Relationships between seed size and hardness of 139 soybean genotypes.
A = GSI10284, B = GSI13125, C = GSI10122,
D = GSI13815, E = GSI10715, F = GSI13833,
G = GSI13866, H = Taekwangkong.

Measurement of seed hardness

Seed hardness was measured 5 times for each genotype using grain hardness tester (Kiya Seisakusho Ltd., Japan).

Determination of water imbibition velocity

Moisture-equilibrated seeds were soaked into distilled water at 25 °C. Seeds were recovered at 0, 0.25, 0.50, 1.0, 2.0, 4.0, 6.0, 8.0, 16.0, 24.0, 36.0, and 48.0 hr after soaking, respectively. After removing surface water by paper towel, imbibed seed weight was measured. The imbibition rate was measured based on the dry weight.

Standard germination test

This test was conducted using standard procedures as described in the "International Rules for Seed Testing" of the International Seed Testing Association (2006). Eight × 50 seed replications were germinated on rolled towel paper at 25 °C for 8 days and classified into the normal, abnormal seedlings, hard seed, and dead seeds. In practice, hard seed are defined here as those that do not absorb enough water in 72 hours.

Measurement of electroconductivity

Seeds were placed into a prepared flask containing distilled water after measuring weight. Each flask was gently swirled to ensure that all seeds were completely immersed. The flask was covered with aluminum foil prior to placing at 25 ± 2 for 24 hours. The conductivity of the soak solution

Table 1. Morphological characteristics of seeds of selected eight soybean genotypes.

Genotypes	Seed size (100 seed weight, g)		Hardness (kg/cm^2)		Seed coat color
GSI10284	Small	(11.5)	Low	(28.5)	Brown
GSI13125	Small	(6.8)	Medium	(62.2)	Black
GSI10122	Small	(9.2)	High	(112.6)	Orange
GSI13815	Medium	(29.8)	Low	(40.3)	Yellow
GSI10715	Medium	(28.1)	High	(119.2)	Black
GSI13833	Large	(49.1)	Medium	(62.2)	Black
GSI13866	Large	(44.8)	High	(84.1)	Black
Taekwangkong	Medium	(26.5)	High	(79.5)	Yellow

was measured at the end of the 24 hour (± 15 minutes) soak period. Electroconductivity was measured by EC meter (C 231 Consort, Belgium).

$$\text{Conductivity } (\mu S\ cm^{-1}\ g^{-1}) = \frac{\text{Conductivity } (\mu S\ cm^{-1}) - \text{background conductivity of the water } (\mu S\ cm^{-1})}{\text{Weight (g) of seed sample}}$$

Complex stressing vigor test

This test was conducted using standard procedures as described ISTA vigor test method (ISTA, 1999). The seed was soaked in distilled water for 48 hours at 25 followed by another 48 hours soaking at 5 °C. Removed seeds from the soak water, seeds were blot dried and germinated on rolled towel paper at 25 °C for 8 days.

Aging treatment

Seeds of eight soybean samples were artificially aged using the "wire-mesh" tray procedure by ISTA vigor test method (1999). Plastic box (10.0 × 10.0 × 3.0 cm) in which a copper wire mesh tray was placed 2.0 cm above the bottom of the box was used for treatment. Forty mL of water were added to each box and 400 seeds were placed on the wire tray. Each box incubated at 41 ± 0.3 and near 100% relative humidity for 72 hrs. Seeds were then allowed to dry at room temperature to have a 10 ~ 14% moisture content.

Aged seeds were subjected to standard germination test and the measurement of electroconductivity described above.

Pot trial

Twenty five aged seeds were sown at 1.5 - 2 cm depth on pots packed with field soil. The pots were watered with same amount of tap water and hold for 24 hr before sowing. Pots were placed in greenhouse. After emergence, seedlings including epicotyl, hypocotyl, and roots were collected and dried to measure dry weight.

RESULTS AND DISCUSSION

Water absorption rate and seed hardness

Water absorption rate

Seed of GSI13125 (Hard seed group, Fig. 1) rarely absorbed water during imbibition and seeds of GSI10284, GSI10122, and GSI10715 were imbibed more slowly than that of GSI13815, GSI13833 and Taekwangkong (Fig. 2). However, final amount of water imbibed into seeds was nearly similar between the two groups. Small and colored seed, GSI10284 (brown), GSI10122 (yellow), and medium and colored seed, GSI10715 (black), showed poor imbibition. This result was similar to the previous report by Park *et al.* (1996) in which water imbibition velocity of black-color soybean seed was lower than yellow coated soybean seed.

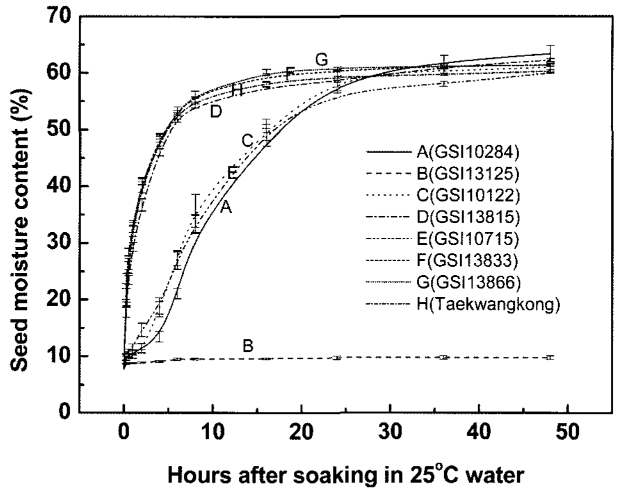

Fig. 2. Changes of seed moisture content during soaking in water of 25°C. Vertical bars represent standard errors of the means.

Hardness

Hardness of unsoaked seed was higher in GSI10122 (112.6 kg cm^{-2}), GSI10715 (119.2 kg cm^{-2}), GSI13866 (84.1 kg cm^{-2}), Taekwangkong (79.5 kg cm^{-2}), moderate in GSI-13125 (62.2 kg cm^{-2}) and GSI13833 (62.2 kg cm^{-2}), and lower in GSI10284 (28.5 kg cm^{-2}) and GSI13815 (40.3 kg cm^{-2}), respectively (Table 1). After soaking in water at 25 for 72 hr, unimbibed hard seed rate was higher in GSI10284, GSI13125, GSI10715. This result suggests that there was no significant correlation between seed hardness and hard seed rate. However, there was a significant negative correlation between seed size and hard seed rate at 1% probability level (Table 7).

Germinability and electroconductivity of unaged seeds

Standard germiantion test

Standard germination test (SGT) was conducted to evaluate the viability of unaged seeds. All genotypes showed higher seed viability than 90% (Table 2). GSI13833 showed the highest abnormal seedling rate (30%) and the rate of Taekwangkong was 13%. Hard seed rate of GSI13125, GSI10715, and GSI10284 were 89, 54, and 42%, respectively, reflecting relatively higher value. The hard seeds, however, maintained viability based on the staining by tetrazolium test.

Table 2. Variations in germinability, hardness and viability of non-aged seed of eight soybean genotypes in standard germination test (SGT) and complex stressing vigor test (CSVT).

Genotypes	Normal seedling (NS[†])	Abnormal seedling (ABS)	Dead seed (DS)	Hard seed (HS)	Seed viability	
					Hard seed	Non-hard seed
SGT	%					
GSI10284	48bc[‡]	8b	2b	42c	98a	83ab
GSI13125	8d	0	3b	89a	97a	73bc
GSI10122	95a	4b	1b	0	99a	95a
GSI13815	97a	3b	0	0	100a	97a
GSI10715	39c	5b	2b	54b	98a	85ab
GSI13833	61b	30a	8a	1d	92b	62c
GSI13866	89a	7b	4ab	0	96ab	89ab
Taekwangkong	85a	13b	2b	0	98a	85ab
CSVT						
GSI10284	51a	5c	4d	40b	96a	85a
GSI13125	9b	2c	2d	87a	98a	69ab
GSI10122	4b	77a	19b-d	0	81bc	4d
GSI13815	42a	49b	9cd	0	91ab	42bc
GSI10715	35a	6c	24b	35b	76c	54ab
GSI13866	0	54b	46a	0	54d	0
Taekwangkong	33a	45b	22bc	0	78bc	33c

Seed viability = percent viable seed plus hard seed after 8 days.
[†]NS = 100 – (ABS + DS + HS).
[‡]mean followed by common letter(s) in a column are not significantly different at 5% level by DMRT.

$$\text{Non-hard seed} = \frac{\text{NS} \times 100}{100 - \text{HS}}$$

Table 3. Normal seedling rates of non-aged seed of eight soybean genotypes in standard germination test (SGT) and complex stressing vigor test (CSVT).

Genotypes	Normal seedling rate, SGT (A)	Normal seedling rate, CSVT (B)	A - B
	%		
GSI10284	83ab[†]	85a	-2
GSI13125	73bc	69ab	4
GSI10122	95a	4d	91
GSI13815	97a	42bc	55
GSI10715	85ab	54ab	30
GSI13833	62c	-	-
GSI13866	89ab	0	89
Taekwangkong	85ab	33c	52

$$\text{Normal seedling rate} = \frac{100 - (\text{abnormal seedling} + \text{dead seed} + \text{hard seed})}{100 - \text{hard seed}} \times 100.$$

[†]mean followed by common letter(s) in a column are not significantly different at 5% level by DMRT.

Electroconductivity

Electroconductivity (EC) of leachate from soaked seed reflects the integrity of seed. Therefore, we investigated the relationship between electroconductivity and storability and hardness of seed. EC of GSI10284, GSI13125, and GSI10715 that showed higher hard seed rate was lower as 10.9, 7.9, and 11.7 μScm^{-1}, respectively. The EC of genotypes showing moderate hard seed rate were higher than that of genotypes showing higher hard seed rate. The EC of GSI13833, GSI13866, Taekwangkong that showed lower hard seed rate were 26.3, 26.5, and 22.5 $\mu S\ cm^{-1}$. This result was similar with the report by Park *et al.* (1996) in which black soybean was coated with a large amount of wax and showed slower imbibition rate and lower EC than yellow soybean. Because the cellular membrane of seeds that have low storability are feasible to be destroyed and the cellular contents can be leached easily, we can assumed that GSI10284 and GSI13125 are higher storability than other genotypes tested.

Complex stressing vigor test

In the complex stressing vigor test (CSVT), seed viability was different greatly among genotypes, GSI10284 (96%),

GSI13125 (98%), GSI10122 (81%), GSI13815 (91%), GSI10715 (76%), GSI13866 (54%), Taekwangkong (78%) (Table 2). The difference of normal seedling rate of non hard seeds between SGT and CSVT was -2 and 4 in GSI10284 and GSI13125, respectively. These two genotypes that showed high hard seed rate were less affected by complex stress imposed during germination. Therefore, it was suggested that these two genotypes have excellent storability under adverse storage condition (Table 3).

Germinability and electroconductivity of aged seeds

Standard germination test

In standard germination test, normal seedling rate of GSI10284, GSI13125, GSI10122, GSI13815, GSI10715, GSI13833, GSI13866 and Taekwangkong was 86, 86, 90, 94, 71, 56, 72 and 73%, respectively (Table 4). The genotypes that showed higher normal seedling rate, GSI10284, GSI13125, GSI10122 and GSI13815, maintained their seed viability under artificial aging treatment. In pot experiment, seedling emergence rate of GSI10122 was drastically decreased to 52% suggesting a low storability. Therefore, it was assumed that the storability of GSI10122 was less than GSI10284, GSI13125 and GSI10715 that have higher hard seed rate.

Pot trial

GSI10284 and GSI13125 showed poor emergence in pot trial due to the relatively high degree of hard seed rate.

Table 4. Germination response of eight soybean genotypes to the accelerated aging treatment in laboratory test and pot trial.

Genotypes	Lab. germination test						Pot trial		
	Normal seedling (NS†)	Abnormal seedling (ABS)	Dead seed (DS)	Hard seed (HS)	Seed viability	$\frac{NS \times 100}{100 - HS}$	Emergence	Shoot length	Seed vigor
	%							(cm)	
GSI10284	63bc‡	6d	4bc	27b	96ab	86ab	58c	33.9a	1,968bc
GSI13125	6d	0	1c	93a	99a	86a	12d	20.4b	245d
GSI10122	89a	8cd	2c	1c	98a	90a	52c	22.8b	1,185cd
GSI13815	94a	6d	0	0	100a	94a	92a	32.4a	2,984ab
GSI10715	55c	19b	3bc	23b	97ab	71c	86ab	32.7a	2,815ab
GSI13833	56c	31a	13a	0	87c	56d	80ab	34.5a	2,763ab
GSI13866	72b	22ab	6a-c	0	94a-c	72c	92a	36.4a	3,349a
Taekwangkong	73b	17bc	10ab	0	90bc	73bc	68bc	31.9a	2,167a-c

Seed viability = percent of viable seed plus hard seed.
Seed vigor = hypocotyl length plus radicle length × percent normal seedling.
NS† = 100 – (ABS + DS + HS).
‡mean followed by common letter(s) in a column are not significantly different at 5% level by DMRT.

Table 5. Normal seedling rate and seed viability of eight soybean genotypes in standard germination test (SGT), complex stressing vigor test (CSVT) and accelerated aging (AA) test.

Genotypes	Normal seedling rate			Seed viability		
	SGT	CSVT	AA	SGT	CSVT	AA(lab)
	%					
GSI10284	83ab†	85a	86ab	98a	96a	96ab
GSI13125	73bc	69ab	86a	97a	98a	99a
GSI10122	95a	4d	90a	99a	81bc	98a
GSI13815	97a	42bc	94a	100a	91ab	100a
GSI10715	85ab	54ab	71c	98a	76c	97ab
GSI13833	62c	-	56d	92b	-	87c
GSI13866	89ab	0	72c	96ab	54d	94a-c
Taekwangkong	85ab	33c	73bc	98a	78bc	90bc

$$\text{Normal seedling rate} = \frac{100 - (\text{abnormal seedling} + \text{dead seed} + \text{hard seed})}{100 - \text{hard seed}} \times 100.$$

Seed viability = 100 – dead seed.
†mean followed by common letter(s) in a column are not significantly different at 5% level by DMRT.

GSI10715 having higher hard seed rate, however, showed high emergence rate. Emergence rate of GSI10122 that has no hard seed was the lowest after aging. Therefore, this genotype might be considered as the lowest genotype in seed storability.

Electroconductivity

Electroconductivity was measured on the leachate from soaked seeds after artificial aging. EC of GSI13125, GSI10284 and GSI10715, the genotypes showing higher hard seed rate, was 7.12 μS cm^{-1}, 18.8 μS cm^{-1} and 18.9 μS cm^{-1}, respectively, after artificial aging (Table 6). EC of hard seed genotypes show similar result with the report by Kwon *et al.*(1987) that EC of soybean leachate was lower in small and black seed soybean among fifty Korean soybean land races. In general, EC of leachate was higher in the artificially aged seeds than unaged seeds. The difference of EC between aged and unaged seed was great in GSI10122 that was assumed as a poor genotype for storage (Table 5).

The correlation between hardness, hundred seed weight, and hard seed rate was presented in Table 7. There was a negative correlation between hundred seed weight and water absorption rate until 24 hr after soaking by which time moisture equilibrium was attained. This result suggests that small seed was slower than large one in germination due to the relatively greater proportion of seed coat which is a primary barrier to water absorption. Our result was consisted with the report of Ragus (1987) in which hard seed was slow in germination because they are small and have thick seed coat.

Seed hardness was negatively correlated with seed viability in CSVT, however, there was no significant relationship

Table 6. Electroconductivity of aged and non-aged seed of eight soybean genotypes.

Genotypes	Non-aged seed (A)	Aged seed (B)	B - A
	----------	mS cm^{-1} g^{-1}	----------
GSI10284	10.9±1.22†	18.8±1.34	7.9
GSI13125	7.9±2.63	7.1±1.25	-0.8
GSI10122	18.9±0.66	30.2±2.79	11.3
GSI13815	16.0±0.02	21.8±0.44	5.8
GSI10715	11.7±1.48	18.9±1.17	7.2
GSI13833	26.3±0.56	32.8±1.15	6.5
GSI13866	26.5±2.01	25.0±1.27	-1.5
Taekwangkong	22.5±2.57	26.6±0.80	4.1

†mean ± standard error.

Table 7. Correlation coefficients between 100 seed weight, hardness, seed germinability and water absorption rate of seed.

Variable	Hardness	100 seed weight	Hard seed (%, SGT)
Hardness	-	0.086	-0.114
100 seed weight	0.086	-	-0.557*
Hard seed (%, SGT)	-0.114	-0.557*	-
Seed viability (SGT)	-0.001	-0.621**	0.076
Conductivity	0.267	0.773**	-0.845**
Seed viability (CSVT)	-0.714**	-0.701**	0.151
Seed viability (aged-lab)	-0.284	-0.742**	0.189
Emergence rate (aged-pot)	-0.470	0.071	-0.665**
Conductivity (aged)	0.277	0.556*	-0.898**
Water absorption 0.5 hr	-0.068	0.801**	-0.724**
〃 4 hr	-0.028	0.836**	-0.790**
〃 16 hr	0.105	0.671**	-0.868**
〃 24 hr	0.113	0.502*	-0.805**
〃 36 hr	0.252	0.359	-0.880**

*, **symbols are significant at 5% and 1% probability level, respectively.

with other characteristics. Although seed hardness is a trait that is easily measured, this trait is affected by several characteristics like the composition of seed coat, cotyledons, and moisture content. In general, the hardness of well dried seed is greater than less dried seed. Therefore, the degree of hardness may reflect the status of changeable viability of seed over time. From the above results, it was deduced that two characters, hundred seed weight and hard seed rate, are closely related to the seed storability. In conclusion, water absorption property of soybean seed is highly related to the seed hardness and the seed hardness is closely related to the seed storability.

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