

Modifying Controlled Deterioration for Evaluating Field Weathering Resistance of Soybean

Ye Changrong^{1,2}, Prapa Sripichitt^{2*}, Sunanta Juntakool²,
Vipa Hongtrakul³ and Arom Sripichitt⁴

ABSTRACT

To develop practical methods for testing field weathering resistance of soybean varieties, pods and seeds from CM60 (susceptible) and GC10981 (resistant) were tested by seven treatments. Among the treatments, modified incubator weathering (yellow pods were incubated at 30°C under 90-100% relative humidity for 7 days) and the controlled deterioration (dry seeds were soaked in distilled water for 60 minutes and then incubated at 41°C under 90-100% relative humidity for 3 days) showed wide-range differences in seed germination and viability between CM60 and GC10981. These two treatments were then tested on 11 soybean varieties comparing with a field weathering treatment. The germination of seeds treated by controlled deterioration was highly correlated to the germination of seeds subjected to field weathering treatment ($r=0.964^{**}$, $n=11$). The viability of seeds submitted to both incubator weathering and controlled deterioration were also correlated to the viability of seeds exposed to field weathering ($r=0.697^*$ and 0.716^* , $n=11$). The modified incubator weathering and controlled deterioration methods were further used to evaluate the field weathering resistance of 139 F₂ progenies derived from the cross CM60/GC10981. There was a significant correlation between the incubator weathering and the controlled deterioration by considering the germination and viability of seeds (germination $r=0.331^{**}$, viability $r=0.425^{**}$, $n=139$). Both the modified incubator weathering and controlled deterioration were efficient for evaluating the field weathering resistance of soybean varieties. Particularly, controlled deterioration method was found to be a useful way for evaluating the field weathering resistance of soybean seeds.

Key words: *Glycine max* (L.) Merr., field weathering resistance, incubator weathering, controlled deterioration

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is one of the world's leading sources of vegetable

oil and plant protein. As the world demand for vegetable oil and protein meal continues to increase, soybean production has spread rapidly from the temperate zone into the hot and humid

¹ Present address: School of Land and Food Science, The University of Queensland, Brisbane, Queensland, Australia.

² Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.

³ Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.

⁴ Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

* Corresponding author: e-mail: agrprs@ku.ac.th

tropics. Following the expansion, weather conditions have become the major factor affecting the soybean seed quality and production in tropical and subtropical regions (Tekrony *et al.*, 1980). Weather conditions (mainly high temperature and relative humidity) during the post-maturation and pre-harvest period increase the difficulty in producing soybean seed with high quality in the tropics. This obstacle in producing good quality seed is also the most important factor that limits a distribution of soybean production in the tropics.

Soybean seed attains its highest vigor, viability and potential quality at physiological maturity (maximum seed dry weight). However, due to high moisture content at physiological maturity (about 55%), the seed cannot be harvested commercially at this stage and must remain on the plant through a desiccation period till the seed reaches a harvestable moisture level. This period may vary from a few days to over 3 weeks depending on the environmental conditions in the field. The seeds deteriorate rapidly during this period (Delouche, 1980). Deterioration of seed vigor, as well as viability, due to high temperature and high relative humidity between the stages of seed physiological maturity and harvesting is referred to as field weathering (Tekrony *et al.*, 1980). Improving the field weathering resistance of new varieties is an important objective for soybean breeding programs in the tropics.

To evaluate the field weathering resistance of soybean varieties, various methods have been developed (Kueneman, 1982; Dassou and Kueneman, 1984; Horlings *et al.*, 1994). Among these methods, delayed harvest and incubator weathering have been widely used for field weathering evaluation. However, it is difficult to unify the maturation time of different varieties to make them suffer the same weather damage by delayed harvest (Kueneman, 1982). Dassou and Kueneman (1984) compared three weathering treatments and concluded that the incubator weathering treatment (incubated at 30°C under

90-95% relative humidity for 10 days) minimized intraplant variability and environmental effects among genotypes with different maturity. The incubator weathering method promised a practical screening procedure for identification of resistance to field weathering of soybean seeds and has been widely used since then. However, the incubator conditions are conducive to the rapid growth of pathogens which is likely to encourage deterioration (Balducchi and McGee, 1987). Horlings *et al.* (1994) modified the treatment to incubate the pods at 27°C under 90-100% relative humidity for 4 days and indicated that the modified incubator treatment had the most detrimental effect on seed quality. But this treatment is probably too gentle since the temperature in tropical soybean fields is normally hotter and the duration is longer.

The field weathering resistance of soybean is usually evaluated by the germination and vigor of the weathered seeds. Controlled deterioration method has been widely used to evaluate seed vigor and viability of seeds (Matthews, 1980; Powell and Matthews, 1981), but the relationship between field weathering and controlled deterioration has not been studied. The purpose of this study is to evaluate the possibility and efficiency of using controlled deterioration treatment for evaluating the field weathering resistance of soybean seeds.

MATERIALS AND METHODS

Plant materials

Twelve soybean varieties/lines, namely Chiangmai 60 (CM60), Yodson, TGX814-26D, Kalitor, 9520-21, 9519-1, Jakapan-1, Lee, CM 9501-3-17, MK-35, SSR 8502-14-1 and GC10981, and 139 F₂ progenies from the cross CM60 / GC10981 were used in this study. Soybean CM60 and GC10981 were employed as susceptible and resistant control for field weathering resistance, respectively (Kaowanant, 2003).

Methods

Experiment A

Soybean CM60 and GC10981 were grown in a field at the National Corn and Sorghum Research Center, Nakhon Ratchasima Province. Water, fertilizer, pesticide and fungicide were applied when necessary. The yellow pods at physiological maturity were harvested for field weathering test. The field weathering resistance was evaluated by seven treatments as follows.

1. Incubator weathering: Fresh yellow pods (36 pods for each treatment) were placed upright in the cells of a grid to avoid pod contact, and then sealed in a plastic box with 1 cm water under the grid to ensure a high relative humidity (90-100%) during the incubation. The boxes with pods inside were incubated by three treatments as follows:

- 30°C for 10 days
- 35°C for 7 days
- 30°C for 7 days

After the incubation, the pods were dried, threshed and the seeds were used for germinating evaluation.

2. Accelerated ageing test and controlled deterioration: Fresh yellow pods were dried and threshed. The seeds (50 seeds for each treatment) were subjected to the following four treatments:

- Seeds were put in a wire-mesh tray. The trays were then sealed in a plastic box with 1 cm water under the trays to ensure a high relative humidity (90-100%) during the incubation. The boxes with seeds inside were incubated at 41°C for 3 days (standard AA test).

- Seeds were soaked in distilled water for 15 minutes, and then put in wire-mesh tray and sealed in a plastic box with 1 cm water under the trays. The boxes with seeds inside were incubated at 41°C for 7 days.

- Seeds were soaked in distilled water for 30 minutes, and then put in wire-mesh tray and sealed in a plastic box with 1 cm water under

the trays. The boxes with seeds inside were incubated at 41°C for 4 days.

- Seeds were soaked in distilled water for 60 minutes, and then put in wire-mesh tray and sealed in a plastic box with 1 cm water under the trays. The boxes with seeds inside were incubated at 41°C for 3 days.

After the treatment, the seeds from each treatment and 50 non-treatment seeds (control) were germinated between wet papers at 25°C for 5 days. The normal seedlings, abnormal seedlings, fresh ungerminated seeds, hard seeds and dead seeds were counted (AOSA, 2000). The field weathering resistance of the variety was evaluated by germination (percentage of normal seedlings and hard seeds) and viability (percentage of normal seedlings, abnormal seedlings, fresh ungerminated and hard seeds) of the treated seeds.

Experiment B

Soybean seeds of CM60, Yodson, TGX814-26D, Kalitor, 9520-21, 9519-1, Jakapan-1, Lee, CM 9501-3-17, MK-35, and *SSR 8502-14-1* were grown in a greenhouse at the Department of Agronomy, Kasetsart University, Bangkok. Water, fertilizer, pesticide and fungicide were applied when necessary. At physiological mature stage, the yellow pods were subjected to the following treatments:

1. Control (no treatment): The yellow pods were harvested, dried and threshed, and then 50 seeds of each variety/line were germinated and investigated as described in experiment A.

2. Field weathering: The yellow pods were left on the plant (green and brown pods were cut out) for 2 weeks with water spraying twice a day. Then the pods were harvested, dried and threshed. Fifty seeds of each variety/line were germinated and investigated as described in experiment A.

3. Incubator weathering: The yellow pods (36 pods for each variety/line) were harvested and placed upright in the cells of a grid, and then

sealed in a plastic box with 1 cm water under the grid. The boxes with pods inside were incubated at 30°C for 7 days. After the treatment, the pods were dried and threshed, and fifty seeds of each variety/line were germinated and investigated as described in experiment A.

4. Controlled deterioration: The yellow pods were harvested, dried and threshed. After measuring the moisture content (MC, wet weight basis) of the seeds, fifty seeds from each variety/line were weighed and soaked in distilled water for 60 minutes, and then the seeds were quickly dried by tissue paper, weighed again and put in a wire-mesh tray. The trays with seeds inside were sealed in a plastic box with 1 cm water under the trays to ensure a high relative humidity (90-100%) during the incubation. The boxes were then incubated at 41°C for 3 days. After the treatment, the seeds were weighed, germinated and investigated as described in experiment A.

Experiment C

Soybean seeds of CM60, GC10981 and their F₂ progenies were grown in a field at the National Corn and Sorghum Research Center, Nakorn Rachasima Province. Water, fertilizer, pesticide and fungicide were applied when necessary. The pods at physiological maturity were harvested from each plant for field weathering evaluation by incubator weathering and controlled deterioration test:

1. Incubator weathering: Fresh yellow pods (18 pods for each progeny) were placed upright in the cells of a grid, and then sealed in a plastic box with 1 cm water under the grid. The boxes with pods inside were incubated at 30°C for 7 days. After the treatment, the pods were dried and threshed, and then the seeds were germinated and investigated as described in experiment A.

2. Controlled deterioration: The yellow pods were harvested, dried and threshed. Twenty-five seeds from each progeny were soaked in distilled water for 60 minutes, and then the seeds

were put in a wire-mesh tray. The trays with seeds inside were sealed in a plastic box with 1 cm water under the trays. The boxes were then incubated at 41°C for 3 days. After the treatment, the seeds were germinated and investigated as described in experiment A.

Data analysis

The frequency distribution, paired t-test and Pearson correlation test of germination and viability data were carried out following the procedure of Komez and Komez (1984).

RESULTS

The efficiency of different treatments

To determine the optimum treatment for evaluating the field weathering resistance of soybean varieties, the seed quality of CM60 and GC10981 was evaluated by seven treatments. The germination and viability of soybean seeds of CM60 and GC10981 after being subjected to seven treatments (experiment A) are shown in Table 1. For incubator weathering, after the pods were incubated, the germination of CM60 and GC10981 seeds were 0-9.2% and 0-42.9%, and the viability of CM60 and GC10981 seeds were 19.5-41.5% and 27.3-72.5%. The most serious seed deterioration was caused by incubating the pods at 30°C for 10 days due to a serious pathogen infection. The seeds of both varieties/lines had lost their germinability (0% germination). Decrease in both treating temperature and time could increase the germination and viability of the treated seeds. After incubating the pods at 30°C for 7 days, the difference between CM60 and GC10981 in germination (33.7%) and viability (31.0%) were greater than the other two treatments. Therefore, this treatment was considered to be more efficient to distinguish the seed weathering of CM60 and GC10981 than the other two treatments.

For the standard accelerated aging (AA) test, the germination of CM60 and GC10981 were

Table 1 The germination and viability of the soybean seeds of CM60 and GC10981 after being subjected to various treatments.

Treatment ¹	Germination (%) ²			Viability (%)		
	CM	GC	Dif.	CM	GC	Dif.
IW: 30°C, 10 days	0.0	0.0	0.0	19.5	27.3	7.8
IW: 35°C, 7 days	6.6	15.2	8.6	35.6	46.8	11.2
IW: 30°C, 7 days	9.2	42.9	33.7	41.5	72.5	31.0
CD: Water 15 min. + 41°C, 7 days	8.0	32.0	24.0	52.0	60.0	8.0
CD: Water 30 min. + 41°C, 4 days	28.0	48.0	20.0	64.0	76.0	12.0
CD: Water 60 min. + 41°C, 3 days	32.0	70.0	38.0	68.0	85.0	17.0
AA: 41°C, 3 days	60.0	84.0	24.0	92.0	100.0	8.0
Control (no treatment)	92.0	94.0	2.0	100.0	100.0	0.0

¹ IW= incubator weathering, CD= controlled deterioration, AA= accelerated ageing

² CM= CM60, GC= GC10981, Dif.=difference= GC-CM.

decreased from 92% to 60% and from 94% to 84%, respectively, but the viability was only decreased slightly from 100% to 92% for CM60 comparing to the control. For controlled deterioration treatments, soaking the seeds in distilled water prior to incubation further decreased the germination and viability of the treated seeds. The lowest germination and viability were caused by soaking the seeds in distilled water for 15 minutes and incubating at 41°C for 7 days. Soaking the seeds in distilled water for 60 minutes and then incubating at 41°C for 3 days showed the widest difference in seed germination (38.0%) and viability (17.0%) between CM60 and GC10981. Thus this treatment was considered to be an optimum one to distinguish the seed weathering of CM60 and GC10981.

If ignore the fault treatment (incubator weathering at 30°C for 10 days), by comparing the means of the other 6 treatments (paired t-test), there was significant difference between CM60 and GC10981 ($t_5=5.825$, $p=0.002$ for germination and $t_5=4.083$, $p=0.01$ for viability). It was clear that the field weathering resistance of CM60 and GC10981 was significantly different.

Relationship among field weathering, incubator weathering and controlled deterioration

To confirm the possibility of using the modified controlled deterioration method for evaluating the field weathering resistance of soybean seed, field weathering (delayed harvest with water spraying) along with incubator weathering and controlled deterioration were carried out on 11 soybean varieties/lines. The seed characters and the changes in moisture content during controlled deterioration treatments are shown in Table 2. The seed moisture content of all the varieties/lines increased (3.12 to 31.1%) after soaking in distilled water for 60 minutes. The moisture content continued to increase during incubation. After soaking and incubation, the seed moisture content increased from 15.72 to 31.25% depending on the variety/line. The final moisture content of all the treated seeds varied from 23.45 to 37.95%. Different varieties absorbed water and moisture at different speeds. The seed moisture content after soaking was highly correlated to the final moisture content ($r=0.951^{**}$, $n=11$) and the final moisture content increase ($r=0.950^{**}$, $n=11$). The increase in moisture content after soaking was also correlated to the final moisture content increase ($r=0.952^{**}$, $n=11$). The treated seeds that absorbed water faster also showed the higher increases in moisture content finally. There was a significant negative correlation between the

moisture content increment after soaking and the moisture content increase after incubation ($r = -0.958^{**}$, $n=11$). The treated seeds that absorbed more water during soaking absorbed less moisture during incubation. In contrast, the treated seeds that absorbed less water during soaking absorbed more moisture during incubation.

The germination, viability and hardseedness of 11 soybean varieties/lines after being subjected to 3 different treatments (field weathering, incubator weathering and controlled deterioration) are shown in Table 3. The germination and viability of seeds decreased after being subjected to all the three treatments

Table 2 The seed characters and changes in moisture content during controlled deterioration treatment of 11 soybean varieties/lines.

Variety/line	Seed color	100 seeds weight (g)	MC* before soaking (%)	MC after soaking (%) (increase)	MC after incubation (%) (increase)
CM60	Yellow	15.11	7.04	30.33 (23.29)	31.63 (24.59)
Yodson	Black	12.13	7.20	15.47 (8.27)	26.76 (19.57)
TGX-814-26D	Yellow	9.47	7.16	22.29 (15.13)	30.83 (23.67)
Kalitor	Black	7.47	7.73	10.85 (3.12)	23.45 (15.72)
9520-21	Yellow	15.74	7.02	32.51 (25.49)	34.09 (27.07)
9519-1	Yellow	11.73	6.70	37.80 (31.10)	37.95 (31.25)
Jakapan-1	Yellow	11.84	7.05	15.95 (8.91)	25.78 (18.73)
Lee	Yellow	8.62	7.21	33.64 (26.43)	35.20 (27.99)
CM9501-3-17	Yellow	8.44	7.18	11.87 (4.69)	28.09 (20.91)
MK35	Yellow	10.25	7.40	21.66 (14.26)	29.94 (22.54)
SSR8502-14-1	Black	13.28	7.13	13.90 (6.77)	24.84 (17.71)

* MC = moisture content

Table 3 The germination, viability and hardseedness of 11 soybean varieties/lines after being subjected to 3 different treatments.

Variety/line	Germination (%)*				Viability (%)				Hard seeds (%)			
	CK	FW	IW	CD	CK	FW	IW	CD	CK	FW	IW	CD
CM60	86	44	30	50	90	70	46	56	4	12	0	0
Yodson	96	66	56	82	98	86	78	86	6	10	6	4
TGX-814-26D	90	62	82	76	96	78	98	88	0	0	0	0
Kalitor	98	76	68	96	100	90	94	100	24	52	12	34
9520-21	96	58	80	72	98	86	96	90	0	0	0	0
9519-1	96	54	56	64	98	84	82	68	0	0	0	0
Jakapan-1	98	56	58	70	98	76	64	80	22	26	0	8
Lee	90	56	68	66	92	80	94	86	0	0	0	0
CM9501-3-17	94	72	64	90	96	88	78	92	14	38	0	8
MK35	92	50	42	68	96	74	62	82	0	0	0	0
SSR8502-14-1	90	62	78	84	100	86	94	96	4	14	2	4

* CK = check (no treatment), FW = field weathering, IW = incubator weathering, CD = controlled deterioration.

comparing to the control. There was a significant correlation between the germination of seeds treated by field weathering and by controlled deterioration ($r=0.964^{**}$, $n=11$), as well as the viability of seeds subjected to these two treatments ($r=0.716^*$, $n=11$). The correlation between the germination of seeds treated by field weathering and by incubator weathering was not obvious. However, there was a significant correlation between the viability of seeds treated by field weathering and by incubator weathering ($r=0.697^*$, $n=11$). To a certain extent, the controlled deterioration treatment was more efficient for indicating the field weathering resistance of soybean than the incubator weathering treatment. The correlation between the germination of seeds treated by incubator weathering and by controlled deterioration was not obvious. There was only a correlation between the viability of seeds treated by incubator weathering and by controlled deterioration ($r=0.739^{**}$, $n=11$). All the tested varieties/lines showed higher germination and viability than CM60. Among these varieties/lines, Kalitor, a variety with black seed coat and high percentage of hardseedness, showed high germination and viability in every treatment. It is a useful resource for future breeding programs of soybean field weathering resistance.

Application of incubator weathering and controlled deterioration on F₂ progenies

The field weathering resistance of 139 F₂ plants was investigated by incubator weathering and controlled deterioration treatment. For incubator weathering treatment, the seed germination of the F₂ progenies ranged from 21.3 to 81.6%, whereas those of their parents, CM60 and GC10981, were 34.7% and 75%, respectively. The viability of the F₂ progenies varied from 47.8 to 95.6%, whereas those of CM60 and GC10981 were 59.7% and 93.4%, respectively. For controlled deterioration test, the germination of the F₂ progenies extended from 20 to 82%,

whereas those of CM60 and GC10981 were 32% and 72%, respectively. The viability of the F₂ progenies ranged from 44 to 90%, whereas those of CM60 and GC10981 were 54% and 94%, respectively. The distribution of the germination and viability of the F₂ progenies are shown in Figure 1. The germination and viability of the seeds under both treatments showed normal distribution (skewness $< \pm 0.5$, kurtosis $< \pm 0.1$). There was a significant correlation between the incubator weathering and the controlled deterioration as assessed by germination ($r=0.331^{**}$, $n=139$) and viability ($r=0.425^{**}$, $n=139$). Both treatments could be used for evaluating the field weathering resistance of soybean seeds.

DISCUSSION

Since the field weathering occurs under the hot and humid conditions in the field after the seeds are physiologically mature, the most common procedure for evaluating seed resistance to field weathering is to leave the plants in the field beyond the normal harvest period and then assess the quality of the seed by visual score, examining seed-borne fungi, seed vigor, or use a combination of these assessment methods. This delayed harvest technique for evaluating the field weathering has several limitations, for example, genotypes matured at different times are subjected to different environmental weathering stresses and different periods of weathering. It is difficult to apply the same environmental stress conditions to cultivars of different maturities by delayed harvest. In an attempt to overcome the limitations of delayed harvest, Kueneman (1982) developed spreader row and overhead irrigation techniques to accelerate weathering based on the delayed harvest method and found the cultivar differences were highly significant. Artificial seed weathering methods, such as incubator weathering, can minimize the effects of variable pod maturity. In

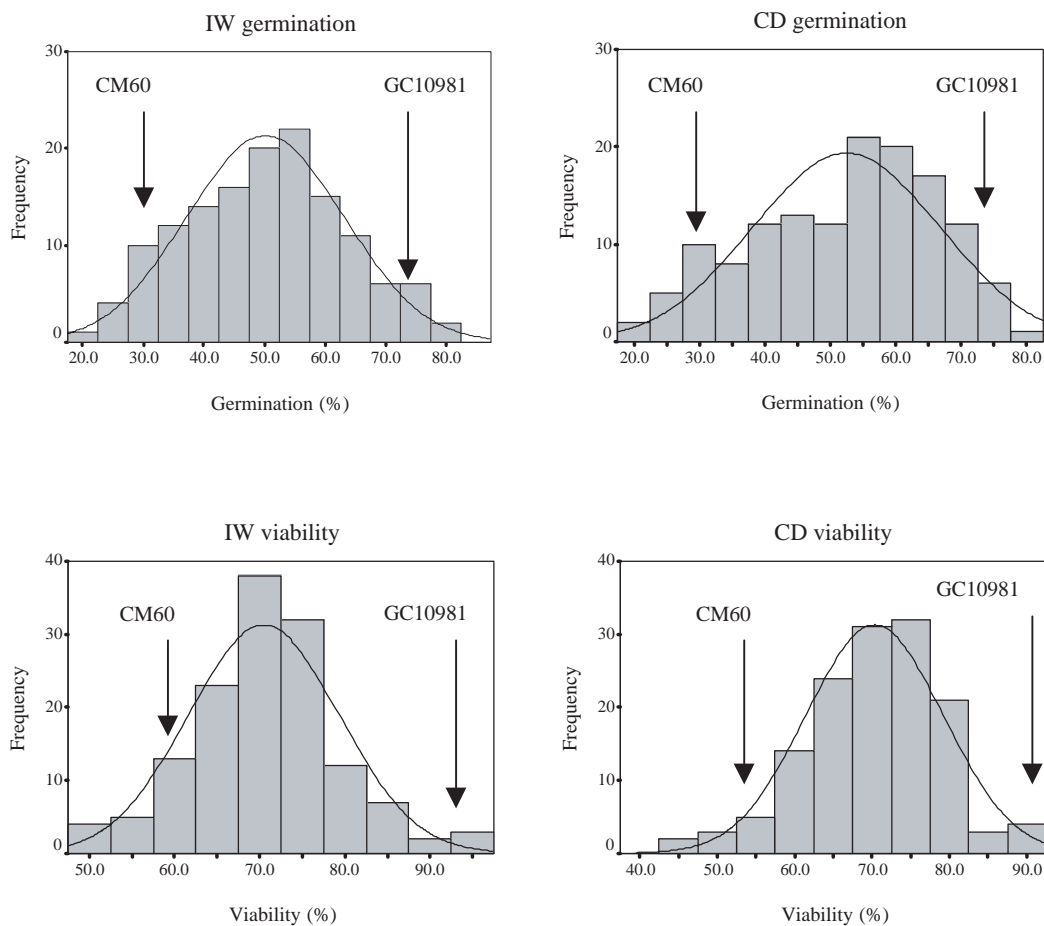


Figure 1 The distribution of seed germination and viability of the F_2 progenies after being subjected to incubator weathering (IW) and controlled deterioration (CD) test. The skewness and kurtosis for each distribution are as follows: IW germination (0.079, -0.523), CD germination (-0.319, -0.742), IW viability (0.199, 0.656), CD viability (-0.395, 0.721).

experiment A of this study, three incubator weathering treatments were carried out to identify the difference between susceptible variety CM60 and resistant variety GC10981. After the fresh yellow pods were incubated at 30°C and 90-100% relative humidity for 10 days, a serious pathogen infection occurred, and some seeds even germinated during the incubation. The remaining seeds had lost their germinating ability (0% germination) and showed a very low viability (19.5% and 27.3%). Increasing the temperature and shortening the incubating time (35°C, 7 days)

reduced the pathogen infection and germination during incubation, but the treatment still caused serious damage to the seeds. This treatment had been successfully used to identify the field weathering difference between CM60 and GC10981 by Kaowanant (2003). However, the constant temperature at 35°C practically does not occur in the soybean field. By reducing the temperature (30°C, 7 days), the pathogen growth and seed germination during incubation were controlled. The results showed obvious differences in germination and viability between CM60 and

GC10981 which could be used to identify the field weathering resistance of these varieties.

On the other hand, since the ability of seed coat to absorb moisture from the environment is a decisive factor in field weathering, the faster the seed absorbs moisture from the environment, the more serious the weathering that occurs. Thus, the controlled deterioration method developed by Matthews (1980) was modified to evaluate the seed weathering. The modified treatments emphasized the relationship between seed moisture absorbing speed and seed weathering. The original controlled deterioration method was modified into three combinations of water soaking time and incubating time to compare with the standard accelerated aging test. Soaking the seeds in distilled water for 60 minutes and incubating at 41°C under 90-100% relative humidity for 3 days showed a wide-ranging difference in germination and viability between CM60 and GC10981. Since the difference in field weathering resistance of these two soybean varieties had been stated by Kaowanant (2003), the treatments which showed more difference between these varieties should be more efficient for distinguishing the field weathering resistance of soybeans. Thus, this treatment was considered to be efficient for testing field weathering resistance of soybean.

The efficiency of the modified incubator weathering and controlled deterioration were further confirmed on 11 soybean varieties/lines in experiment B. Highly significant correlation was found between the germination of seeds treated by field weathering and by controlled deterioration ($r=0.964^{**}$, $n=11$), as well as the viability of seeds subjected to these two treatments ($r=0.716^*$, $n=11$). It is possible to use this controlled deterioration method to predict the field weathering resistance of soybean varieties. There was highly significant negative correlation between the water absorbing speed and the final germination of the treated seeds ($r=-0.785^{**}$, $n=11$), it was confirmed that the seeds absorbing

water faster would suffer more serious deterioration during the incubation resulting in a lower percentage of germination. The incubator weathering and controlled deterioration methods were further applied to evaluate the field weathering resistance of F_2 progenies derived from the cross CM60/GC10981. The germination and viability of seeds subjected to both treatments were continuous with a normal distribution. There was highly significant correlation between incubator weathering and controlled deterioration by considering germination and viability of the seeds (germination $r=0.331^{**}$, viability $r=0.425^{**}$, $n=139$). Both incubator weathering and controlled deterioration may be used to determine the field weathering resistance of soybean varieties. However, it is difficult to treat a great number of pods at the same time in incubator weathering test due to the laboratory limitations, especially in large-scale breeding programs. Controlled deterioration method makes it possible to harvest the pods at physiological maturity, dry to a similar moisture content level, and then store for testing. This will be very beneficial to large-scale screening in breeding programs.

CONCLUSION

The modified controlled deterioration (soaking seeds in distilled water for 60 minutes and incubating at 41°C under 90-100% relative humidity for 3 days) was confirmed to be useful for evaluating field weathering resistance of soybean seeds based on the hypothesis of a correlation between the water absorbing speed and field weathering resistance of seeds, especially for large-scale soybean breeding programs that focus on seed quality.

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