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Evaluation of Soybean [*Glycine max* (L.) Merrill] Germplasm for Field Weathering Resistance using Seed Quality and SCAR Markers

Nan Pa Pa Win[1], Prapa Sripichitt[2]*, Wanchai Chanprasert[2], Vipa Hongtrakul[3] and Chalermpol Phumichai[2]

ABSTRACT

Forty-eight soybean varieties/lines along with resistance and susceptibility checks were grown at the National Corn and Sorghum Research Center, Nakhon Ratchasima province, Thailand in a randomized complete block design with three replications. At physiological maturity, soybean pods were harvested and subjected to incubator weathering, controlled deterioration and electrical conductivity tests to evaluate the seed quality. Soybean seed physical characteristics, including seed coat percentage, seed weight and seed coat color were also investigated. Two sequence characterized amplified region (SCAR) primers, Eaag/Mcac-233 and Eact/Mctt-157, previously reported to link with a *quantitative trait locus* (QTL) that controlled field weathering resistance, were used to amplify the DNA of the 50 soybean varieties/lines. The results revealed that 48 soybean varieties/lines were significantly different in seed quality and seed physical characteristics. They were classified into four groups: resistant, moderately resistant, moderately susceptible and susceptible to field weathering, according to the average germination percentage after incubator weathering and controlled deterioration. The overall field weathering resistance was found to correlate with seed quality and seed physical characteristics. The two highest correlation coefficients were found between field weathering resistance and germination percentage after incubator weathering ($r = 0.898^{**}$) and controlled deterioration ($r = 0.888^{**}$). The two SCAR primers were able to differentiate between resistance and susceptibility checks but were unable to classify field weathering resistance in 48 soybean varieties/lines. It was concluded that the two markers were not appropriate for evaluating field weathering resistance in the soybean varieties/lines.

Key words: soybean, physiological maturity, field weathering, seed quality, SCAR marker

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the world's leading sources of vegetable oil and plant protein, which are very well adapted for human nourishment. A major constraint to the expansion of soybean production in the tropics is the difficulty in producing high quality seed. Tropical conditions of high temperature and relative humidity during the seed maturation period are not conducive to the production of high quality seed, which is necessary to establish

1 Department of Agricultural Research, Yezin, Myanmar.
2 Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
3 Department of Genetics, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: agrprs@ku.ac.th

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acceptable stands (Paschal and Ellis, 1978). Deterioration of seed due to high temperature and relative humidity prior to harvest (during maturation) in the field is known as field weathering (TeKrony *et al*., 1980).

In order to evaluate varietal differences in the field weathering resistance of soybean seed, various methods have been developed (Dassou and Kueneman, 1984; Horlings *et al*., 1994) and assessing seed germination, viability and vigor after weathering have been considered meaningful ways (Kueneman, 1982). Delayed harvest and incubator weathering have been widely used as evaluation techniques for field weathering resistance in soybean. A major difficulty in using delayed harvest is the application of the same environmental conditions to cultivars of different maturity. Dassou and Kueneman (1984) compared three weathering methods: 1) field weathering, 2) incubator weathering and 3) wet-bag weathering and reported that incubator weathering minimized intraplant variability and environmental effects among the genotypes with different maturity. However, incubator conditions were conducive to the rapid growth of pathogens, which were likely to encourage deterioration. Therefore, incubator weathering along with some modification was used to evaluate field weathering resistance under tropical conditions (Horlings *et al.,* 1994; Kaowanan, 2003; Marwanto, 2003; Changrong *et al*., 2007a). Changrong *et al*. (2007a) indicated that a modified incubator treatment (incubation at 30° C under 90-95% relative humidity for 7 d) was efficient in evaluating the field weathering resistance of soybean. A controlled deterioration method also has been developed for evaluating seed vigor (Matthews, 1980). Changrong *et al*. (2007a) modified the controlled deterioration treatment to compare the ability of the seed coat to absorb moisture from the environment and found a relationship between controlled deterioration and field weathering resistance. The vigor of soybean seeds could be indirectly determined using the electrical conductivity of seed leachate (Hampton and TeKrony, 1995). High vigor seeds were able to reorganize their membranes more rapidly and repair any damage to a greater extent than low vigor seeds could. The electrolyte leachate measured from the high vigor seeds was less than that measured from the low vigor ones. Kuo (1989) proved that the electrical conductivity test was also an effective screening method for delayed permeability in the seed coat of soybean.

Soybean seeds with an impermeable seed coat were more resistant to field weathering than those with a permeable seed coat (Potts *et al.,* 1978). The seeds with lower permeability had higher specific weights of the testa (Kuo, 1989). Soybean seeds with small seed size tended to have a greater seed coat percentage (Smith and Circle, 1978). Black and small-seeded soybean genotypes were more resistant to weathering than the yellow and large-seeded ones (Nangju, 1977; Dassou and Kueneman, 1984). Cultivars with a black seed coat were found to be adapted to a tropical climate (Dassou and Kueneman, 1984).

At present, DNA markers have been widely utilized for the identification of the genes controlling various characters. Changrong *et al.* (2007b) developed two SCAR markers that were linked to field weathering resistance. The markers were used for assisting the selection of soybean genotypes in backcross progenies that were resistant to field weathering. Since field weathering resistance of soybean has been able to be phenotypically and genotypically identified, breeding programs have had to concentrate on incorporating this into commercially acceptable cultivars.

The objectives of this study were 1) to evaluate soybean varieties/lines for resistance to field weathering using seed quality and SCAR markers and 2) to determine the relationship between field weathering resistance and seed quality, seed physical characteristics and SCAR markers.

MATERIALS AND METHODS

Planting of soybean

Fifty soybean varieties/lines (Table 1) were planted at the National Corn and Sorghum Research Center, Nakhon Ratchasima province in a randomized complete block design with three replications in the 2008 rainy season. Soybean varieties/lines CM60 and GC10981 were used as susceptibility and resistance checks, respectively. The seeds of each variety/line were sown in plots of four rows, each 3 m long, with a spacing of 50 cm between rows and 20 cm between plants within a row. Water, fertilizers, fungicides and insecticides were applied when necessary.

Methods for seed quality test

The yellow pods harvested at physiological maturity were used throughout the test.

Incubator weathering test

Fifty fresh yellow pods were placed upright in the cells of a grid. The grid was sealed in a plastic germination box with water 1cm deep under the grid to ensure a high relative humidity (90-95%) during incubation. Then, the boxes with pods inside were incubated at 30°C for 7 d (Dassou and Kueneman, 1984; modified by Changrong *et al*., 2007a). After 7 d, the pods were removed from the incubator, dried to approximately 12% moisture content and hand-threshed.

Fifty seeds were germinated between wet paper at 25°C for 5 d. The numbers of normal seedlings, abnormal seedlings, fresh un-germinated seeds, hard seeds and dead seeds were counted. The germination percentage was calculated according to ISTA (2004).

Controlled deterioration test

The pods were air dried to approximately 12% moisture content and hand-threshed. Then, 25 seeds were soaked in distilled water for 1 h and put on a wire-mesh tray. The trays were sealed in a plastic box with water 1cm deep under the trays to ensure a high relative humidity (90-100%) during incubation. The boxes were then incubated at 41°C for 3 d (Matthews, 1980; modified by Changrong *et al*., 2007a). The treated seeds were subjected to the same germination test as mentioned previously.

Electrical conductivity test

The pods were air dried to approximately 12% moisture content and hand-threshed. Then, 25 seeds were weighed and soaked in 75 ml deionized water in a 200 ml beaker. A control treatment was used by adding only 75 ml deionized water into the 200 ml beaker. The non-control beakers were covered with aluminum foil and incubated at 20°C for 24 hours. Then, the electrical conductivity (EC) of the seed leachate was recorded in units of microSiemen per cm per gram of seed (µS/cm/gseed).

Measurement of seed physical characteristics

Seed coat percentage

The pods were air dried to approximately 12% moisture content and hand-threshed. Ten seeds were soaked in distilled water and incubated at 5°C for 15-16 h. The seed coat was separated from each seed using a razor blade. The seeds (without seed coats) and the seed coats were dried in a hot air oven at 100°C for 24 h. After drying, the seeds (without seed coats) and seed coats were weighed separately and the seed coat percentage was calculated (Kuo, 1989).

Seed weight

The weight in grams of 100 seeds (12% moisture content) of each of 50 soybean varieties/lines was measured.

Seed coat color

The seed coat color of the 50 soybean varieties/lines was visually observed and recorded.

Evaluation of field weathering resistance

Based on the susceptibility and resistance checks, the field weathering resistance of the 48 soybean varieties/lines was evaluated using the average germination percentage after incubator weathering and controlled deterioration and then

Table 1 Soybean varieties/lines used for evaluation of field weathering resistance.

Variety/line	Origin or parentage	Physiological maturity (days)
SJ 1	Taiwan	95
SJ 2	Japan	100
SJ 4	Acadian × Tainung 4	119
SJ 5	Tainung 4 × SJ 2	93
CM 2	CM 60 × IAC 13	109
CM 3	G9946 × AGS17	111
CM 4	G9946 × AGS17	119
CM 60 (susceptible check)	Williams × SJ 4	100
CM 9123-4	Thailand	85
CM 9238-54-1	Thailand	102
CM 9501-3-17	Thailand	97
CM 9510-1	Thailand	92
CM 9510-5	Thailand	110
CM 9511-4	Thailand	102
CM 9513-1	Thailand	85
ST 1	Shih Shih × SRF 400	110
ST 2	7016 × ST 1	104
ST 3	Fort Lamy × CM 60	102
Nakhon Sawan 1	Doteung × Santa Maria	91
9518-2	Thailand	114
9519-1	Thailand	102
9502-16	Thailand	98
9520-21	Thailand	102
GC 4120	AVRDC, Taiwan	105
GC 4796	AVRDC, Taiwan	90
GC 7231	AVRDC, Taiwan	88
GC 9984	AVRDC, Taiwan	110
GC 10215	AVRDC, Taiwan	100
GC 10848	AVRDC, Taiwan	88
GC 10981(resistant check)	AVRDC, Taiwan	88
GC 11101	AVRDC, Taiwan	85
SSR 8502	Thailand	102
SSR 8407-Y-2-1	Thailand	84
SSR 8412-9-2	Thailand	100
MK 35	Unknown	114
PK 462	Korea	92
M-Pop-8-BL	Thailand	90
KUSL 20004	Clark63 × Orba	88
KKU 35	Williams × SJ 2	103
EHP 275	Thailand	109
PI 205908-2	Thailand	98
PI 205912	Thailand	102
TGx 814-26D	IITA, Nigeria	108
TGx 536-02D	IITA, Nigeria	111
Kalitor	India	105
Damtia 1	Thailand	91
Lee	U.S.A.	112
Fort Lamy	U.S.A.	109
Yoadson	Thailand	91
Beagumhong	Korea	109
LSD(0.05)		29.46

categorized into four groups: resistant, moderately resistant, moderately susceptible and susceptible to field weathering.

Evaluation procedure for SCAR marker

The 50 soybean varieties/lines were sown in seedling trays for DNA extraction. Ten days after emergence, young leaves from each variety/line were sampled and subjected to DNA extraction using the protocol of Doyle and Doyle (1990) with minor modification. The DNA obtained was adjusted to a concentration of 100 ng/μl and stored at -20°C.

Two SCAR markers, Eaag/Mcac-233 and Eact/Mctt-157, developed from AFLP primers by Changrong *et al*. (2007b) were used to amplify the DNA of the 50 soybean varieties/lines. These two markers gave amplification (PCR) products of 233 and 157 bp, respectively (Changrong *et al*., 2007b). The banding patterns of the two SCAR markers for the 50 soybean varieties/lines were recorded.

RESULTS AND DISSCUSSION

Field weathering resistance

Table 2 clearly shows that the 50 soybean varieties/lines were significantly different ($p<0.01$) in germination percentages after incubator weathering and controlled deterioration and consequently they had significantly different ($p<0.01$) in average germination percentages after both weathering tests. The average germination percentage was used to classify the tested varieties/lines as resistant, moderately resistant, moderately susceptible or susceptible to field weathering.

Six varieties/lines (GC 4796, GC 10215, Fort Lamy, GC 10848, Kalitor and GC 11101) were found to be resistant to field weathering. The average germination percentages after incubator weathering and controlled deterioration of these varieties/lines varied from 60.02 to 87.67%. The average germination percentages of GC 4796, GC 10215, Fort Lamy, GC 10848 and Kalitor were higher than that of the resistance check, GC 10981 (64.34%). In particular, GC 4796 produced the highest average germination percentage among the resistant varieties/lines. Therefore, these varieties/lines could be considered more resistant to field weathering than the resistance check. Sixteen varieties/lines were revealed to be moderately resistant to field weathering, as the average germination percentages of this group ranged from 41.34 to 56.67% which were higher than both the overall mean (38.53%) and the susceptibility check, CM 60 (8.00%). Therefore, these varieties/lines were more resistant to field weathering than the susceptibility check. Nineteen varieties/lines were found to be moderately susceptible to field weathering because their average germination percentages varied from 20.50 to 38.84%. The average germination percentages of these varieties/lines were lower than the overall mean (38.53%) but higher than that of the susceptible check (8.00%) except those of CM 9501-3-17 and PI 205908-2 which were higher than the overall mean. Therefore, these varieties/lines were more resistant to field weathering than the susceptiblility check, but they tended to be more susceptible to field weathering than the moderately resistant varieties/lines. Seven varieties/lines were classified as susceptible to field weathering because their average germination percentages ranged from 9.34 to 19.65%, which were the lower values among the tested varieties/lines. Therefore, these varieties/lines could be considered susceptible to field weathering, eventhough their average germination percentages were higher than the susceptibility check (8.00%).

The results showed that resistant varieties/lines exhibited higher average germination percentages after incubator weathering and controlled deterioration than the susceptible ones. The high temperature and humidity that the seeds were subjected to under each treatment reduced the germination of susceptible seeds by seed weathering. The varieties/lines with genetically good seed quality performed well after the weathering conditions. Therefore, it was clear that there were varietal differences in field weathering resistance. Dassou and Kueneman (1984), Kueneman (1982), Kaowanan (2003), Marwanto (2003) and Changrong *et al.* (2007a) used germination percentages after incubator weathering and controlled deterioration to evaluate the field weathering resistance of soybean germplasm and reported that there were varietal differences in resistance to field weathering. Paschal and Ellis, (1978) Ndimande *et al.* (1981) and Nangju (1977) observed that some soybean varieties showed inherently more resistance to field deterioration.

Some seed quality and seed physical characteristics contributed to field weathering resistance

The seed vigor estimated by the EC values of the seed leachate of the 50 soybean varieties/lines is shown in Table 2. The tested varieties/lines were significantly different ($p<0.01$) in seed vigor (EC value of seed leachate), which contributed to the differences in field weathering resistance.

Table 2 Field weathering resistance, seed quality and seed physical characteristics of 50 soybean varieties/lines.

Variety/line	IWG	CDG	Average*	EC	%SC	SW	SC
Resistant							
GC 4796	83.33	92.00	87.67	30.75	6.82	18.42	Green
GC 10215	81.33	68.00	74.67	37.30	7.30	18.54	Yellow
Fort Lamy	73.33	76.00	74.67	38.14	9.05	12.25	Black
GC 10848	91.33	56.00	73.67	48.20	8.69	13.82	Brown
Kalitor	51.30	81.33	66.32	48.18	7.94	12.18	Black
GC 11101	42.70	77.33	60.02	32.07	8.14	13.05	Green
Average	70.55	75.11	72.84	39.11	7.99	14.71	-
Moderately resistant							
Yoadson	53.33	60.00	56.67	51.35	7.56	17.73	Black
SSR 8412-9-2	47.00	66.00	56.50	51.20	7.98	15.25	Black
M-Pop-8-BL	45.33	60.00	52.67	74.00	8.18	12.65	Black
CM 9511-4	78.00	26.67	52.34	53.81	6.66	20.59	Yellow
SSR 8502	48.00	52.00	50.00	59.59	7.75	18.46	Black
ST 3	43.33	54.00	48.67	53.52	7.54	14.75	Black
EHP 275	61.33	36.00	48.67	48.09	6.76	20.07	Yellow
Beagumhong	36.00	58.67	47.34	58.00	6.72	19.55	Yellow
Damtia 1	41.30	51.30	46.30	51.84	7.87	14.99	Black
CM 4	59.00	33.33	46.17	53.02	6.79	17.80	Yellow
SJ 4	56.67	32.00	44.34	58.00	7.33	18.96	Yellow
CM 2	65.33	22.67	44.00	64.32	6.58	20.66	Yellow
GC 7231	44.70	40.00	42.35	37.06	8.26	16.36	Yellow
CM 9123-4	35.33	49.33	42.33	61.73	6.83	19.76	Yellow
Nakhon Sawan 1	43.30	40.00	41.65	52.75	5.65	27.32	Yellow
SSR 8407-Y-2-1	46.67	36.00	41.34	64.18	6.86	16.02	Yellow
Average	50.29	44.87	47.58	55.78	7.21	18.18	-

Table 2 Field weathering resistance, seed quality and seed physical characteristics of 50 soybean varieties/lines (cont).

Variety/line	IWG	CDG	Average*	EC	%SC	SW	SC
Moderately susceptible							
CM 9501-3-17	17.67	60.00	38.84	61.85	7.49	16.20	Yellow
PI 205908-2	32.00	45.33	38.67	54.69	6.36	21.09	Yellow
GC 9984	38.00	36.00	37.00	78.90	7.95	21.03	Yellow
GC 4120	39.67	32.70	36.19	54.90	7.00	21.28	Yellow
TGx 536-02D	47.33	24.00	35.67	51.96	6.44	20.41	Yellow
CM 9238-54-1	23.30	48.00	35.65	54.66	6.65	16.56	Yellow
CM 9510-1	27.33	37.33	32.33	54.36	6.63	19.86	Yellow
CM 9510-5	26.00	33.33	29.67	64.13	6.82	19.29	Yellow
9520-21	25.33	33.33	29.33	53.72	6.44	21.37	Yellow
ST 2	24.00	33.00	28.50	48.71	5.66	21.21	Yellow
KUSL 20004	24.30	28.00	26.15	69.27	8.39	19.78	Yellow
9502-16	18.70	33.33	26.02	54.96	7.19	18.90	Yellow
CM 3	34.67	17.33	26.00	56.41	6.37	18.50	Yellow
PK 462	24.00	25.33	24.67	64.15	6.85	16.69	Yellow
TGx 814-26D	31.00	17.33	24.17	80.65	7.20	15.00	Yellow
Lee	20.70	26.00	23.35	71.85	7.36	13.76	Yellow
PI 205912	8.67	36.00	22.34	67.40	6.05	25.92	Yellow
ST 1	12.00	29.33	20.67	57.31	6.13	20.09	Yellow
SJ 5	11.00	30.00	20.50	54.00	6.78	19.56	Yellow
Average	25.56	32.93	29.25	60.73	6.83	19.29	-
Susceptible							
SJ 2	23.30	16.00	19.65	64.85	6.79	18.33	Yellow
CM 9513-1	18.00	14.70	16.35	67.29	6.69	23.32	Yellow
9519-1	22.00	10.67	16.34	62.35	7.00	20.70	Yellow
9518-2	13.33	13.33	13.33	65.84	6.12	21.81	Yellow
SJ 1	16.30	9.33	12.82	79.92	7.68	17.11	Yellow
MK 35	9.00	16.00	12.50	109.70	9.00	19.70	Yellow
KKU 35	8.00	10.67	9.34	67.70	6.45	20.18	Yellow
Average	15.70	12.96	14.33	73.95	7.10	20.16	-
Check							
GC 10981(resistance)	62.00	66.67	64.34	46.20	7.84	14.60	Green
CM 60 (susceptibility)	6.67	9.33	8.00	70.27	6.09	25.84	Yellow
Overall mean	37.84	39.22	38.53	58.30	7.13	18.55	-
LSD(0.05)	4.22	4.79	0.12	11.23	0.48	1.86	-
CV (%)	6.9	7.5	-	11.9	4.2	6.2	-
P value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	-

IWG = germination percentage after incubator weathering, CDG = germination percentage after controlled deterioration, EC= electrical conductivity of seed leachate (μS/cm/gseed), %SC = percentage of seed coat, SW = seed weight (g/100 seeds), SC = seed coat color.

* average of germination percentage after incubator weathering and controlled deterioration. This was used to classify soybean germplasm as: resistant=above 60%, moderately resistant=40-59.99%, moderately susceptible=20-39.99% and susceptible=below 20%.

The EC values of resistant varieties/lines varied from 30.75 to 48.20 μS/cm/gseed with an average of 39.11 μS/cm/gseed. The EC values of the resistant varieties/lines, except for those of GC 10848 (48.20 μS/cm/gseed) and Kalitor (48.18 μS/cm/gseed), were lower than that of the resistant check, GC 10981(46.20 μS/cm/gseed). Therefore, most of the varieties/lines in this group could be considered to have higher seed vigor than the resistant check. The EC values of the moderately resistant varieties/lines varied from 37.06 to 74.00 μS/cm/g seed with an average of 55.78 μS/cm/gseed, whereas those of the moderately susceptible varieties/lines ranged from 48.71 to 80.65 μS/cm/gseed with an average of 60.73 μS/cm/gseed. The average EC value of the moderately resistant varieties/lines was lower, while that of the moderately susceptible varieties/lines was higher than the overall mean (58.30 μS/cm/gseed). Thus, the moderately resistant varieties/lines could be considered to have higher seed vigor than the moderately susceptible varieties/lines. The EC values of the susceptible varieties/lines varied from 62.35 to 109.70 μS/cm/gseed with the highest average value being 73.95 μS/cm/gseed. The EC values of SJ 2, CM 9513-3, 9519-1, 9518-2 and KKU 35 were lower than that of the susceptibility check. Therefore, these varieties/lines could be considered to have higher seed vigor than the susceptible check. Meanwhile, EC values of SJ 1 and MK 35 were higher than that of the susceptibility check. These two varieties/lines could be considered to have lower seed vigor than the susceptible check.

The results revealed that resistant varieties/lines showed higher seed vigor (lower EC values of seed leachate) than the susceptible ones. This result was in agreement with the study reported by Marwanto (2003) that there was a genotypic difference in resistance to weathering treatments among 11 soybean genotypes and the resistant genotypes showed lower EC values of seed leachate (higher seed vigor) than the susceptible ones. Phan *et al.* (2006) evaluated the F5 lines of two crosses for field weathering resistance and found that the lines with low EC values of seed leachate (higher seed vigor) were resistant to field weathering. Potts *et al.* (1978) reported that the resistant varieties with high seed vigor possessed an impermeable seed coat. Membrane structure and cell leachate were usually associated with seed vigor. The highly vigorous seeds could re-establish their membrane integrity at a faster rate with less leachate (lower EC value) than the less vigorous ones (Hampton and TeKrony, 1995).

The seed physical characteristics including seed coat percentage, seed weight (g/100 seeds) and seed coat color of the 50 soybean varieties/lines are summarized in Table 2. There were significant differences ($p<0.01$) in seed coat percentages among the tested varieties/lines. The seed coat percentages of resistant varieties/lines varied from 6.82 to 9.05% with an average of 7.99%. The varieties/lines of Fort Lamy, GC 10848, Kalitor and GC 11101 had higher seed coat percentages than the resistant check (7.84%). In particular, Fort Lamy had the highest seed coat percentage (9.05%) among the resistant varieties/lines. The seed coat percentages of the moderately resistant varieties/lines varied from 5.65 to 8.26% with an average of 7.21%, whereas those of the moderately susceptible varieties/lines ranged from 5.66 to 8.39% with an average of 6.83%. It was observed that the average seed coat percentage of the moderately resistant varieties/lines was higher than that of the moderately susceptible varieties/lines. The seed coat percentage of the susceptible varieties/lines varied from 6.12 to 9.00% with an average of 7.10%. All susceptible varieties/lines showed higher seed coat percentages than the susceptible check. In particular, MK 35 had a much higher seed coat percentage (9.00%) than the resistant check (7.84%), which made the average seed coat percentage of the susceptible varieties/lines (7.10%) higher than that of the moderately susceptible varieties/lines (6.83%).

This result manifested that the resistant varieties/lines had a tendency to produce higher seed coat percentages than the susceptible ones.

This finding was consistent with the result reported by Phan *et al*. (2006) that the F_5 lines with field weathering resistance showed higher seed coat percentages than the susceptible ones. Horling *et al*. (1991) stated that the resistance to field weathering in soybean was associated with seed coat characteristics.

There were significant differences (p<0.01) in seed weights among the tested varieties/lines (Table 2). The seed weight of the resistant varieties/lines varied from 12.18 to 18.54g with an average of 14.71g per 100 seeds. Some varieties/lines, such as Fort Lamy, GC 10848, Kalitor and GC 11101 had a lower seed weight than the resistant check (14.60g). In particular, the Kalitor variety had the lowest seed weight among the resistant varieties/lines. The seed weight of the moderately resistant varieties/lines varied from 12.65 to 27.32g with an average of 18.18g, while those of the moderately susceptible varieties/lines ranged from 13.76 to 25.92g with an average of 19.29g. The average seed weight of the moderately resistant varieties/lines was lower than that of the moderately susceptible varieties/lines. The seed weight of the susceptible varieties/lines varied from 17.11 to 23.32g with an average of 20.16g. Although the average seed weight of the susceptible varieties/lines was the highest among the four groups, all susceptible varieties/lines had a lower seed weight than the susceptible check (25.84g).

The results indicated that the resistant varieties/lines tended to have lower seed weights than the susceptible ones. The large-seeded genotypes tended to be highly susceptible to weathering compared with the small-seeded ones because the large-seeded genotypes tended to have a low percentage of hard seed and usually had poor seedling emergence following field weathering and incubator weathering (Edwards and Hartwig, 1971; Dassou and Kueneman, 1984). Chanprasert *et al*. (2000) reported that seeds with a low weight tended to have a high seed coat percentage. The results from the current study also revealed that soybean genotypes with a low weight tended to have a high seed coat percentage, which caused greater resistance to field weathering.

Black, green, brown and yellow seed coat colors were found among the resistant varieties/lines (Table 2). The moderately resistant varieties/lines, including Yoadson, SSR 8412-9-2, M-Pop-8-BL, SSR 8502, ST 3 and Damtia 1, possessed a black seed coat, while the remainder had a yellow seed coat. The seed coat color of all moderately susceptible and susceptible varieties/lines was yellow. The seed coat color of the resistant check was green, whereas that of the susceptible check was yellow. Ndimande *et al*. (1981) found that the black-seeded accessions of soybean were more resistant to field weathering than the yellow-seeded ones. Dassou and Kueneman (1984), Starzinger and West (1982) and Marwanto (2003) reported that the black-seeded type consistently exhibited a greater resistance to all weathering treatments than the yellow-seeded ones, with a higher mean germination and germination after accelerated aging and a lower mean EC value of seed leachate. The current study results showed that not all resistant varieties/lines had a black seed coat. This finding was similar to the result reported by Marwanto (2003) that the yellow-seeded genotypes were superior to some black-seeded ones based on germination after accelerated aging, which was possibly due to their smaller seed size and the higher lignin content in the seed coat. It was concluded that seed coat color was not a mechanism of resistance to field weathering.

SCAR markers analysis for field weathering resistance

Figure 1 shows the DNA amplification of the 50 soybean varieties/lines using SCAR markers (Eaag/Mcac-233 and Eact/Mctt-157). Both markers were able to identify the resistance check (GC10981) with no band and the susceptible check (CM60) with a band across the 50 soybean varieties/lines. However, some resistant varieties/lines showed bands and some susceptible ones exhibited no band using these two markers. In order to analyze the association of the markers and

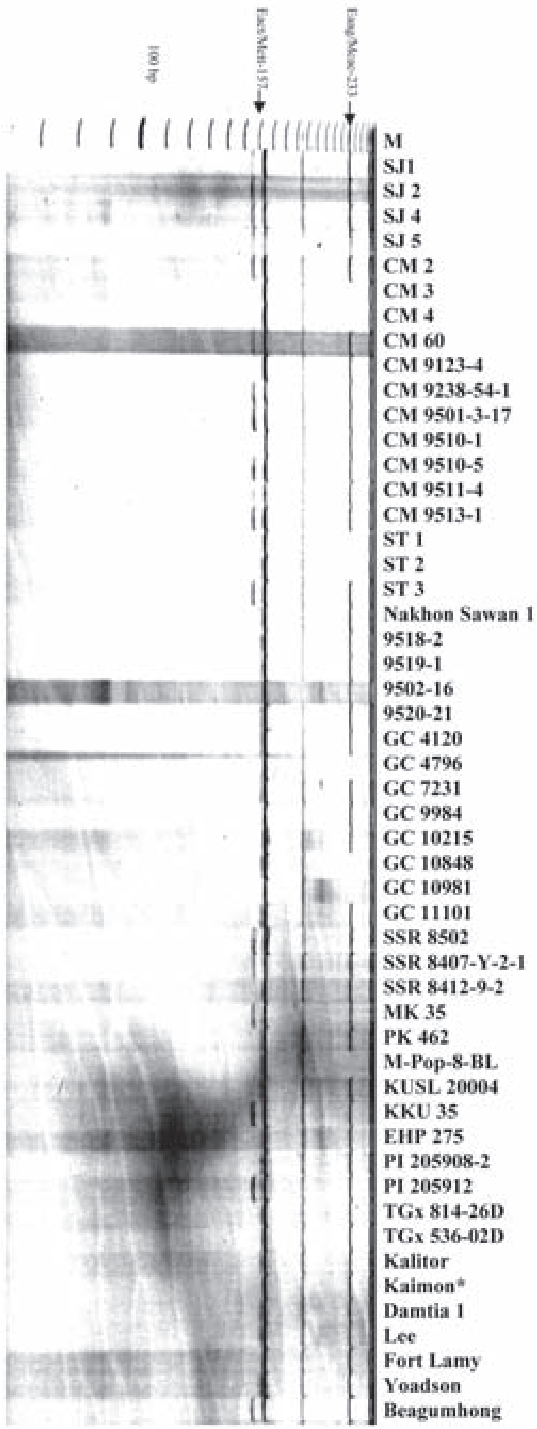

Figure 1 DNA amplification of 50 soybean varieties/lines using two SCAR primers (Eaag/Mcac-233 and Eact/Mctt-157), lane 8 (CM 60) and lane 30 (GC 10981) are susceptibility and resistance checks, respectively.

* Variety was not tested for field weathering resistance.

M: 10 bp DNA ladder (Invitrogen)

field weathering resistance, the correlation between the markers and field weathering resistance was investigated, but no correlation was found. The genotypic expression of the two markers was not consistent with the phenotypic expression (field weathering resistance). A major QTL for field weathering resistance was identified between these two markers (Changrong *et al.*, 2006). However, the genetic distance between both markers was 25.8 cM, which meant that both markers were not tightly linked to a major QTL for field weathering resistance. A marker at 1 centimorgan (cM) indicates a 1% recombination between the marker and the QTL. If the QTL were linked completely to the marker locus, all variation contributed by the QTL would be associated with the differences between marker genotypes. Moreover, if marker-assisted selection were to be of maximum utility, recombination between marker alleles and QTL would need to be as near zero as possible. With a QTL that is associated with many loci or a small number of major genes, it is not possible to focus on individual loci with the degree of accuracy that can be brought to bear on quantitative markers. Thus, markers closely linked to QTL are needed (Dudley, 1993). Arahana *et al.* (2001) reported that markers tightly linked to resistant genes would help to identify resistant soybean lines on the basis of the genotype and could maximize the effectiveness of selection. Babu *et al.* (2004) pointed out a number of constraints on efficient utilization of QTL-mapping information in plant breeding. The salient constraints were: 1) a limited number of major QTL controlling specific traits had been identified, 2) a requirement of the additional QTL identification whenever different sets of breeding material were used and 3) the lack of the application of universally valid QTL-marker associations for different sets of breeding materials.

Relationship between field weathering resistance and seed quality and seed physical characteristics

The correlation coefficients were calculated between field weathering resistance and seed quality and seed physical characteristics (Table 3). The field weathering resistance was positively correlated with germination percentage after incubator weathering and controlled deterioration and with seed coat percentage, and negatively correlated with the EC value of seed leachate and the seed weight. The highest two correlations were found between field weathering resistance and both germination percentage after incubator weathering (r = 0.898**) and germination percentage after controlled deterioration (r = 0.888**). These correlations suggested that soybean varieties/lines with higher percentages of germination and seed coat, and lower EC values of seed leachate and lower seed weight tended to be more resistant to field weathering. This finding was in agreement with the work by Dassou and Kueneman (1984) that found soybean genotypes with a high percentage

Table 3 Correlation coefficients (r) between field weathering resistance and seed quality and seed physical characteristics of 50 soybean varieties/lines.

Trait	Field weathering resistance
IWG	0.898**
CDG	0.888**
EC	- 0.681**
Seed coat percentage	0.412**
Seed weight (g/100 seeds)	- 0.495**

IWG = germination percentage after incubator weathering, CDG = germination percentage after controlled deterioration, EC = electrical conductivity of seed leachate (µS/cm/gseed).
** Significance at the 0.01% level of probability.

of germination following incubator weathering were resistant to field weathering. Marwanto (2003) reported that germination after weathering stress significantly correlated with seed quality during weathering. Chanprasert *et al*. (1996) found that the seed coat percentage and seed weight were correlated with seed quality during field deterioration (weathering). The results from the analysis of seed coat color and seed weight in this study were consistent with the results reported by Dassou and Kueneman (1984) and Nangju (1977) that black and small seeded (low seed weight) genotypes were more resistant to seed weathering than yellow and large seeded (high seed weight) ones.

CONCLUSION

The 48 soybean varieties/lines showed variation in field weathering resistance and they were classified into resistant, moderately resistant, moderately susceptible and susceptible groups according to the average germination percentage after incubator weathering and controlled deterioration. The field weathering resistant varieties/lines were GC 4796, GC 10215, Fort Lamy, GC 10848, Kalitor and GC 11101, while the susceptible ones included SJ 2, CM 9513-1, 9519-1, 9518-2, SJ 1, MK 35 and KKU 35. Seed germination percentages after incubator weathering and controlled deterioration were found to be the most useful parameters for evaluating field weathering resistance compared to other parameters. The varieties/lines with field weathering resistance had high germination percentages, low EC values of seed leachate (high seed vigor), high seed coat percentages and relatively small seeds. Not all of the black-seeded varieties/lines exhibited field weathering resistance. Some yellow, green and brown-seeded varieties/lines with relatively small seed sizes also showed resistance to field weathering. The color of the seed coat could not be used to identify field weathering resistance. The two SCAR markers, which were not tightly linked to the QTL that controlled field weathering resistance, could not distinguish between the resistant and susceptible varieties/lines of the soybean germplasm.

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