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Breeding of Soybean (*Glycine max* (L.) Merrill) for Field Weathering Resistance by Pedigree Method

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ABSTRACT

Breeding of soybean for field weathering resistance was conducted by hybridization between commercial variety CM60 which is susceptible to field weathering and two field weathering resistant varieties (GC2796 and SJ1) in 2003. The F_1 hybrid plants were grown in the greenhouse at the Department of Agronomy, Kasetsart University and the F_2 to F_4 progenies were planted in the field at National Corn and Sorghum Research Center, Nakhon Ratchasima province. Field weathering resistance of soybean was evaluated from some seed characteristics involving seed germinability after accelerated aging, seed vigor estimated from the electrical conductivity of seed leachates, percentage of seed coat and seed coat thickness. Selection was done in each succeeding generation from F_2 to F_4 using pedigree method for plants/lines which had high seed germinability and vigor, high percentage and thickness of seed coat and good agronomic characters. The yield trial of 17 F_5 lines was performed at National Corn and Sorghum Research Center in rainy season 2005. Six F_5 lines having high seed yield, field weathering resistance and good agronomic characters were selected. These promising lines will be further evaluated for seed yield, field weathering resistance and agronomic performance in different locations.
Key words: soybean, pedigree method, field weathering, seed vigor

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) has been an important cultivated crop of the world for many centuries. It is one of the world's leading sources of vegetable oil and plant protein both of which are very well adapted to nourishment of human beings (Scott and Aldrich, 1983). Soybean is orginated in the northern China where environmental stresses were relatively minimal. However, as the world demand for vegetable oil and protein continues to increase, soybean production has been spread rapidly into the hot and humid areas, and more recently into the tropical regions (TeKrony *et al*., 1980). A major obstacle to the expansion of soybean production to new areas of the tropics is the difficulty in producing high quality seed. Tropical conditions of high temperature and relative humidity during the final stages of seed maturation are not conducive to production of high quality seed necessary to establish acceptable stands (Paschal and Ellis, 1978). The process of deterioration in seed quality occurs between the stages of the post-

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maturation and pre-harvest period is referred to as field weathering.

The quality of soybean seed at harvest time depends heavily on the field weathering conditions during the development, maturation and storage of the seeds on the plant. Field weathering of soybean seeds can be overcome by adjusting the planting date to avoid raining period which may occur during the stage of physiological maturity and pre-harvest period or using the field weathering resistant variety. Especially, field weathering resistant variety allows the farmers to grow soybean in both rainy and dry seasons. Pascal and Ellis (1978) and Ndimande *et al*. (1981) reported that varietal differences had been identified for resistance to field weathering and deterioration during storage. Some seed characteristics were found to be very beneficial under tropical conditions (resistant to field weathering) such as hard seed coat (Hartwig and Potts, 1987), small seed size (Nangju, 1979), black seed coat (Dassou and Kueneman, 1984) and hardseed (Delouche, 1975). TeKrony *et al*. (1980) found that one of the main factors appeared to contribute to the low vigor soybean seed was the highly permeable seed coat through which the seed absorbed moisture easily and thus tended to be more susceptible to weathering in the field as well as to humid tropical environment under open storage conditions. However, no report has dealt with the breeding of soybean for field weathering resistance.

The objective of this study was to breed soybean variety which could resist to field weathering by hybridizing CM60, a commercial but susceptible to field weathering variety, with two field weathering resistant varieties (GC2796 and SJ1) and selection using pedigree method.

METERIALS AND METHODS

1. Hybridization and selection

Hybridization was made between commercial variety CM60 which was susceptible to field weathering and field weathering resistant varieties (GC2796 and SJ1) (Kaowanant, 2003) in 2003. The F_1 hybrid plants were grown in the greenhouse at the Department of Agronomy, Kasetsart University, Bangkok. The F_2 progenies were planted in the field at the National Corn and Sorghum Research Center, Pakchong district, Nakhon Ratchasima province. Sixty one and sixty eight F_2 plants from the crosses CM60/GC2796 and CM60/SJ1 were selected for field weathering resistance and good agronomic characters. The selection of the F_3 and F_4 progenies from both crosses was conducted at the National Corn and Sorghum Research Center using pedigree method for lines which manifested field weathering resistance and good agronomic characters. Seventeen F_5 lines of both crosses were grown for yield test using randomized complete block design with 4 replications. Yield test was done at the National Corn and Sorghum Research Center during rainy season 2005. Seed yield (kg/ha) was calculated at 12 percent moisture content. Data of some agronomic characters and yield components were averaged on 10 plants/line.

2. Evaluation of field weathering

Soybean seeds of F_2 to F_5 progenies were evaluated for field weathering resistance. Pods in yellow color were separated from plants at physiological maturity and dried in air until the pods turn brown and dry. The seeds were threshed by hand from the pods and dried in air for 1-2 days (about 12% moisture content). Field weathering of soybean seeds was determined using 3 following tests:

2.1 Accelerated aging test (AA tests)

Fifty seeds with two replications were placed 4 cm above the water surface and incubated at 40°C and 100% relative humidity for 3 days. After incubation, seed germinability was evaluated by laboratory germination test (ISTA, 1985).

2.2 Electrical conductivity test

Twenty five seeds were weighed with two replications and soaked in 75 ml deionized water containing in 200 ml beaker. Control treatment was done by adding only 75 ml deionized water into 200 ml beaker. The beakers were covered with aluminum foil and incubated at 20°C for 24 hours. The electrical conductivity (EC) of mixture (leakage of substances from seeds and deionized water) and deionized water (control treatment) were measured after incubation by electrical conductivity meter. The EC of seed leachates was determined by subtracting the EC of deionized water from the EC of mixture. The EC of seed leachates was recorded in microSeimen (mS) per cm per gram of seed.

2.3 Measurement of seed coat thickness and percentage of seed coat

Ten seeds were soaked in distilled water containing in beaker and incubated at 5°C for 15 – 16 hours. The seed coat was separated from seed using razor blade. Both seed (without seed coat) and seed coat were dried in hot air oven at 105°C for 24 hours. After drying, the seed (without seed coat) and seed coat were weighed. The seed coat thickness was measured using digital vernier. The percentage of the seed coat was calculated by the formula of Kuo (1989) as follows:

% Seed coat = (Seed coat dry weight / Seed and seed coat dry weight) × 100

RESULTS AND DISCUSSION

Field weathering resistance

The soybean plants/lines were selected from the crosses between commercial variety CM60 which was susceptible to field weathering and two field weathering resistant varieties, GC2796 and SJ1. The F_2 progenies were evaluated for field weathering resistance by accelerated aging test (AA test). The F_2 plants of two soybean crosses CM60/GC2796 and CM60/SJ1 were divided into two groups, resistance and susceptible to field weathering. The plants showing seed germination percentage after AA test more than 80% were considered as resistance to field weathering by the suggestion of Vieira *et al*. (2004). There were 273 and 278 resistant plants recovered in the crosses CM60/GC2796 and CM60/SJ1, respectively. Sixty one and sixty eight plants from the crosses CM60/GC2796 and CM60/SJ1 were selected for field weathering resistance and good agronomic characters, respectively. Field weathering resistance of the F_3, F_4 and F_5 lines of both crosses was evaluated from some seed characteristics including seed germinability after AA test, seed vigor estimated from the EC value of seed leachage, percentage of seed coat and seed coat thickness. Seventeen F_5 lines having field weathering resistance and good agronomic characters were screened in the yield test.

All of the F_5 lines showed higher percentage of seed germination (70.5 to 92.5%) than the check variety CM60 (68.5%) which was sensitive to field weathering (Kaowanant, 2003) (Table 1). All of the lines except line no. 3 presented lower EC value (53.01 to 76.98 µS cm^{-1} g^{-1}) than the check variety CM60 (84.93 µS $cm^{-1}g^{-1}$). Soybean lines having EC value ranged from 60 to 70 µS $cm^{-1}g^{-1}$ and 70 to 80 µS $cm^{-1}g^{-1}$ were considered to be high and intermediate vigor seeds (Vieira *et al*., 1994) whereas those possessing EC value higher than 150 µS $cm^{-1}g^{-1}$ were classified as low vigor seeds and assumed to be inadequate for sowing (AOSA, 1983). Seed coat percentage of all lines (6.07 to 8.82%) were higher than that of the check variety CM60 (6.03%). The lines having low EC value or high seed vigor tended to manifest high percentage and thickness of seed coat as verified by the negative correlation between EC value and seed coat percentage (r = -0.8219) and seed coat thickness (r = -0.6671) (Figure 1a, b). Kuo (1989) reported that soybean seed possessing higher specific weight of testa (14.16%) showed lower membrane permeability. Consequently, high seed vigor of soybean lines observed in this experiment might be resulted from the delayed permeability of the seed coat when the seeds exposed to field weathering. This finding opens up the possibility of breeding soybean cultivar resistant to field weathering by improving seed which produces high proportion of seed coat with delayed permeability. The negative correlation between seed germination percentage and EC value found in this experiment (r = -0.7634) (Figure 1c) implied that the seed exhibiting high germination percentage showed high vigor as well. Soybean lines presenting high percentage of seed germination (> 70 %) and vigor (EC value <88 $\mu Scm^{-1}g^{-1}$), high weight (>6 %) and thickness (>0.1 mm) of seed coat were considered to be field weathering resistance.

Fourteen of the 17 F_5 lines were classified to have field weathering resistance higher than CM60, however, their resistance were not higher than the resistant check variety (GC2796). Among them the soybean line no. 1, 2, 4, 14 and 16 (CM60/GC2796-23-2-1, CM60/GC2796-200-34-3, CM60/SJ1-34-6-3, CM60/SJ1-350-49-15, CM60/SJ1-401-55-17) were the top five highest field weathering resistant lines.

Agronomic characters

Some agronomic characters of the F_5 lines of two soybean crosses were investigated.

Table 1 Some seed characters attributed to field weathering resistance of the seventeen F_5 lines in yield test grown at National Corn and Sorghum Research Center in rainy season 2005.

Line no.	Pedigree	Seed germination[2/] (%)	EC ($\mu S\ cm^{-1}g^{-1}$)	Seed coat color	Percentage of seed coat	Seed coat thickness (mm)
1	CM60/GC2796-23-2-1	92.0 abc[3/]	56.44 fgh	Pale Yellow	8.38 a-d	0.158 a
2	CM60/GC2796-200-26-2	88.5 abc	60.35 efg	Pale Yellow	8.13 b-e	0.119 bc
3	CM60/GC2796-225-34-3	70.5 d	87.67 a	Pale Yellow	6.07 h	0.090 cd
4	CM60/SJ1-34-6-3	89.5 abc	57.00 fgh	Yellow	8.54 ab	0.124 abc
5	CM60/SJ1-47-9-4	83.0 c	75.01 c	Yellow	6.92 g	0.101 bc
6	CM60/SJ1-135-21-6	92.5 ab	64.45 dfg	Yellow	7.89 b-e	0.102 bc
7	CM60/SJ1-209-29-8	88.5 abc	68.23 cde	Yellow	7.74 de	0.118 bc
8	CM60/SJ1-209-29-9	88.0 abc	76.98 bc	Yellow	7.83 cde	0.131 ab
9	CM60/SJ1-232-32-10	84.0 bc	62.12 d-g	Yellow	8.44 abc	0.125 abc
10	CM60/SJ1-276-38-11	84.5 bc	63.88 def	Yellow	8.12 b-e	0.116 bc
11	CM60/SJ1-305-42-12	95.5 a	61.62 d-g	Yellow	7.63 ef	0.120 bc
12	CM60/SJ1-314-45-13	85.5 bc	62.72 d-g	Yellow	7.76 de	0.120 bc
13	CM60/SJ1-315-46-14	84.5 bc	71.93 cd	Yellow	7.07 fg	0.118 bc
14	CM60/SJ1-350-49-15	92.5 ab	61.90 d-g	Yellow	8.82 a	0.114 bc
15	CM60/SJ1-356-50-16	89.0 abc	60.82 d-g	Yellow	7.98 b-e	0.109 bc
16	CM60/SJ1-401-55-17	90.0 abc	53.01 gh	Yellow	8.15 b-e	0.128 ab
17	CM60/SJ1-449-61-20	92.0 abc	64.00 def	Yellow	7.56 ef	0.120 bc
18	GC2796[1/]	95.0 a	50.05 h	Pale Yellow	8.29 a-d	0.129 ab
19	SJ1[1/]	90.0 abc	64.35 def	Yellow	7.84 cde	0.115 bc
20	CM60[1/]	68.5 d	84.93 ab	Yellow	6.03 h	0.090 cd
	Mean	87.53	65.37	-	7.76	0.117
	C.V.%	9.9	6.4	-	5.1	18.4

[1/] Parents used as check varieties

[2/] From accelerated aging test

[3/] Means within a column followed by a common letter are not significantly different at 95% level of confidence by DMRT

The number of days to 50% flowering of the F_5 lines ranged from 43 to 46 days and from 38 to 45 days in the crosses CM60/GC2796 and CM60/SJ1, respectively (Table 2). The lines of the cross CM60/SJ1 flowered earlier and showed larger variation in the number of days to 50% flowering than those of the cross CM60/GC2796. The number of days to harvest extended from 89 to 92 days and from 92 to 110 days in the crosses CM60/GC2796 and CM60/SJ1, respectively. Although the F_5 lines of the cross CM60/SJ1 flowered earlier than those of the cross CM60/GC2796, they were harvested later because three of them possessed indeterminate growth habit which was contributed from their male parent SJ1.

The F_5 lines of the cross CM60/GC2796 exhibited very narrow variation in plant height (90 to 91cm) while those of the cross CM60/SJ1 showed wide variation in plant height (78 to 121cm). All lines of the cross CM60/GC2796 possessed higher plant stature than their parents. The tall plant of some F_5 lines from the cross CM60/SJ1 was caused by their stem indetermination which was inherited from their male parent SJ1.

All lines of the cross CM60/GC2796 had

Table 2 Some agronomic characters of the seventeen F_5 lines in yield test grown at National Corn and Sorghum Research Center in rainy season 2005.

Line no.	Pedigree	Days to 50% flowering	Days to harvest	Plant height (cm)	Stem termination	Lodging score[2/]
1	CM60/GC2796-23-2-1	45	89	90	Det.	1
2	CM60/GC2796-200-26-2	43	92	91	Det.	1
3	CM60/GC2796-225-34-3	46	89	90	Det.	1
4	CM60/SJ1-34-6-3	43	96	90	Det.	1
5	CM60/SJ1-47-9-4	43	105	121	Indet.	4
6	CM60/SJ1-135-21-6	43	92	85	Det.	1
7	CM60/SJ1-209-29-8	41	96	94	Det.	1
8	CM60/SJ1-209-29-9	43	96	84	Det.	1
9	CM60/SJ1-232-32-10	38	98	108	Det.	2
10	CM60/SJ1-276-38-11	41	110	115	Indet.	3
11	CM60/SJ1-305-42-12	45	95	98	Det.	1
12	CM60/SJ1-314-45-13	44	109	114	Indet.	4
13	CM60/SJ1-315-46-14	43	98	92	Det.	1
14	CM60/SJ1-350-49-15	43	96	91	Det.	1
15	CM60/SJ1-356-50-16	43	95	89	Det.	1
16	CM60/SJ1-401-55-17	43	95	111	Det.	3
17	CM60/SJ1-449-61-20	43	95	78	Det.	1
18	GC2796[1/]	35	82	32	Det.	1
19	SJ1[1/]	43	110	137	Indet.	4
20	CM60[1/]	43	98	70	Det.	1

[1/] Parents used as check varieties

[2/] Lodging from vertical line was scored on mature soybean plants in a row with ranging from 1 to 5 levels: 1 = all plants in row were erect; 2 = 1-25% of plants in row were prostrate; 3 = 25-50% of plants in row were prostrate; 4 = 51-75% of plants in row were prostrate; 5 = > 75% of plants in row were prostrate erect stem type whereas those of the cross CM60/SJ1 varied in stem type from erect to lodging at level 4. The lodging of soybean plants was caused by tall plant height which was resulted from stem indetermination. This result was supported by the high correlation (r) between plant height and lodging score which was estimated to be 0.9140 (Figure 1d). Similarly, Cooper (1971) reported that cultivars with stem indetermination were generaly taller and lodging more than the stem determination cultivars.

Yield and yield components

Yield and yield components of the 17 F_5 lines of two soybean crosses were determined in yield test done in rainy season 2005. The 17 lines manifested the variation in number of pods per plant from 82.75 to 133.40, the number of seeds per pod from 2.07 to 2.73, 100 seed weight from 13.00 to 19.58g and seed yield from 2652.5 to 4601.5 kg/ha (Table 3). All of the yield components and yield were significantly different among the lines. Eight of the 17 lines gave higher seed yield than the highest yielding check variety (CM60) especially the line no.1 (CM60/SJ1-356-50-16) which exhibited the highest yield of 4601.5 kg/ha.

High yielding of the soybean lines was caused by the high number of pods per plant which was confirmed by the high correlation between

Table 3 Yield and yield components of the seventeen F_5 lines in yield test grown at National Corn and Sorghum Research Center in rainy season 2005.

Line no.	Pedigree	No. of pods/plant	No. of seeds/pod	100-seed weight (g)	Yield (kg/ha)
1	CM60/SJ1-356-50-16	133.40 a[2/]	2.16 c-h	17.99 a-d	4601.5 a
2	CM60/SJ1-350-49-15	124.50 abc	2.09 fgh	18.93 abc	4203.9 ab
3	CM60/SJ1-135-21-6	119.60 a-e	2.24 a-e	17.05 b-f	4111.0 ab
4	CM60/SJ1-315-46-14	122.50 a-d	2.14 d-h	16.03 d-h	4095.1 ab
5	CM60/SJ1-305-42-12	119.67 a-e	2.21 b-g	19.27 a	4078.5 ab
6	CM60/SJ1-232-32-10	107.82 b-g	2.27 a-d	18.41 abc	3964.9 bc
7	CM60/SJ1-34-6-3	122.85 a-d	2.32 ab	15.85 e-h	3874.2 bcd
8	CM60/GC2796-200-26-2	102.07 d-h	2.22 a-f	18.42 abc	3822.5 bcd
9	CM60/SJ1-209-29-9	99.75 e-h	2.2 b-h	18.45 abc	3415.3 def
10	CM60/SJ1-449-61-20	96.75 fgh	2.25 a-e	17.89 a-e	3180.5 efg
11	CM60/GC2796-225-34-3	95.92 gh	2.16 c-h	19.58 a	3110.5 efg
12	CM60/SJ1-209-29-8	96.08 gh	2.16 c-h	16.87 c-f	3089.8 efg
13	CM60/SJ1-276-38-11	95.42 gh	2.18 b-h	15.39 f-i	3007.1 efg
14	CM60/JS1-47-9-4	82.75 h	2.12 e-h	14.39 g-i	2983.2 efg
15	CM60/GC2796-23-2-1	87.65 gh	2.07 gh	18.77 abc	2916.7 fg
16	CM60/SJ1-401-55-17	118.17 a-f	2.37 a	13.49 ij	2854.8f g
17	CM60/SJ1-314-45-13	90.00 gh	2.32 ab	13.08 j	2652.5 g
18	GC2796[1/]	55.17 i	2.24 a-e	16.27 d-g	1459.7 h
19	SJ1[1/]	127.50 ab	2.29 abc	14.03 hij	3239.9 ef
20	CM60[1/]	113.67 c-h	2.07 h	19.11 ab	3500.9 cde
	Mean	105.56	2.20	17.06	3408.1
	C.V.%	12.80	3.9	7.6	10.1

[1/] Parents used as check varieties

[2/] Means within a column followed by a common letter are not significantly different at 95% level of confidence by DMRT

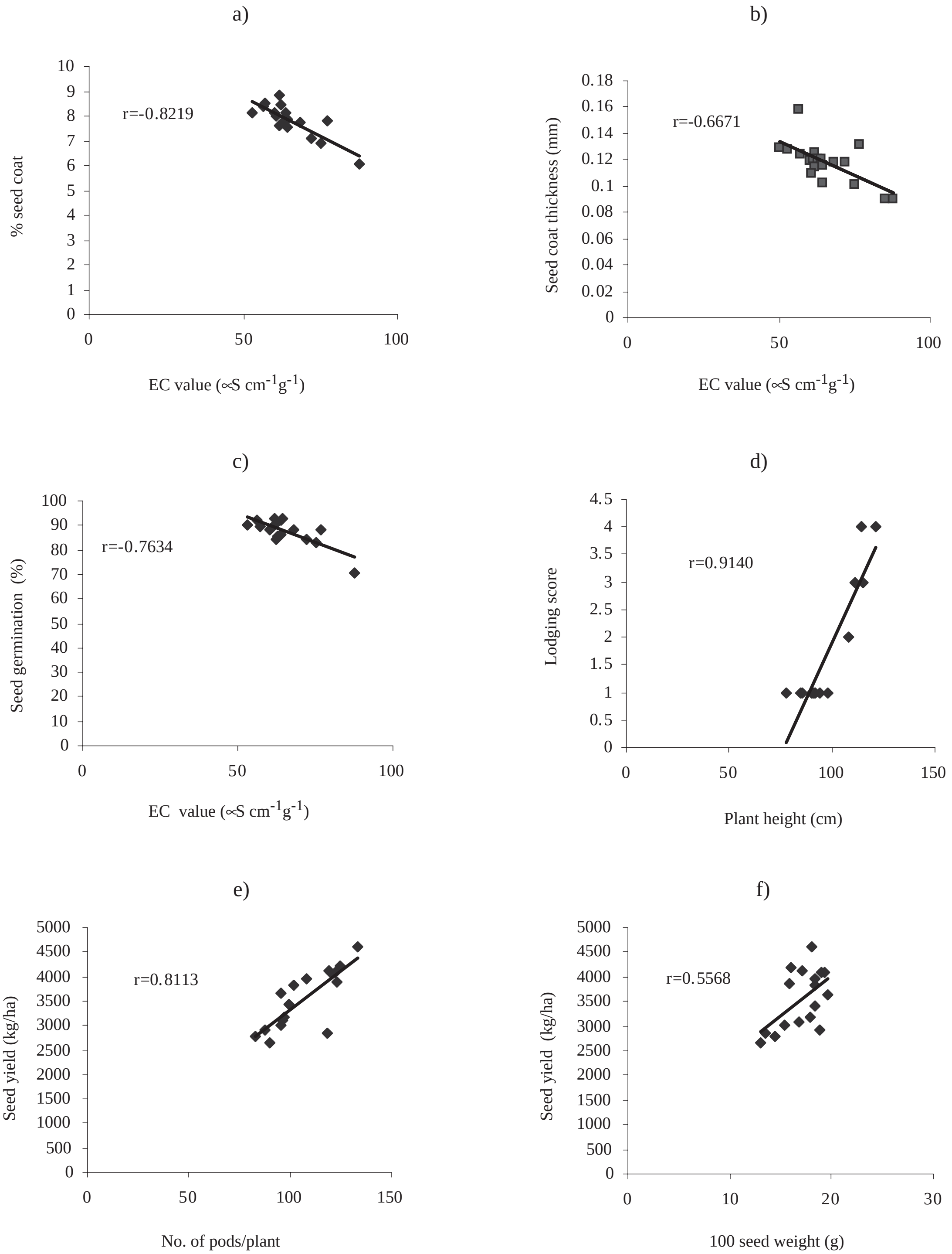

Figure 1 Correlation between some characters of 17 F_5 soybean lines: a) EC value and percentage of seed coat b) EC value and seed coat thickness c) EC value and seed germination percentage d) plant height and lodging score e) number of pods/plant and seed yield f) and 100 seed weight and seed yield.

yield and number of pods per plant (r = 0.8133) (Figure 1e). This observation was in agreement with Verawudh (1974) and Shrivastava (1977) who reported that the highest yielding cultivars of soybean were derived from the cultivars producing the highest number of pods per plant. Correlation between yield and 100 seed weight was relatively low (r = 0.5568) (Figure 1f) so that yield was slightly affected by the seed weight. Similar observation has been made by Egli *et al.* (1978) and Hartwig and Edwards (1970) who found that soybean yield was not closely related to seed size or weight but it was highly correlated with seed number.

Seed yield was also influenced by the type of stem termination where stem determinated lines yielded higher than stem indeterminated ones (Table 2 and 3). Stem determination minimizes the overlap of vegetative and reproductive growth. Thus, a greater proportion of post-anthesis photosynthates can be available to reproductive organs and consequently to increase seed yield (Lin and Nelson, 1988). Lodging could also be a limitation to maximum seed yield in stem indeterminated soybean cultivar. Therefore, some breeding efforts have been directed towards the development of short-stature, determinated cultivar to reduce lodging (Cooper, 1971; Beaver and Johnson, 1981).

CONCLUSION

Six advanced F_5 lines of soybean including one line from the cross CM60/GC2796 (CM60/GC2796-200-26-2) and five lines from the cross CM60/SJ1 (CM60/SJ1-34-6-3, 135-21-6, 305-42-12, 350-49-15 and 356-50-16) were selected based on high yielding, field weathering resistance and good agronomic characters. These lines will be further evaluated for field weathering resistance, seed yield and agronomic performance in different locations.

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