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Premature Harvest of Soybean Seed for Rapid Generation Advance[1,2]

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ABSTRACT

The harvest of immature seeds was investigated as a means of enhancing rapid generation advance in soybean [*Glycine max* (L.) Merr.] breeding programs. One objective of the study was to determine field emergence of immature seeds harvested from genotypes adapted to temperate climates when grown in tropical environments. A second objective was to compare pod color and the ratio of seed width (SW) to pod width (PW) and SW to pod thickness (PT) as indicators of the time to harvest immature viable seed. Two cultivars were planted in three environments in the Iowa State University Soybean Nursery at the Isabela Research Center of the University of Puerto Rico. Harvest of seeds began 24 days after flowering (DAF) and continued at weekly intervals until 59 DAF. Two harvest procedures were compared: removing pods from plants, and pulling plants without detaching the pods. Field emergence, pod color, PW, PT, and SW were measured for each harvest procedure, harvest date, cultivar, and environment. There were no significant differences between harvest procedures for average field emergence. Significant differences were observed among environments, cultivars, and harvest dates. The harvest of immature seed 31 DAF resulted in adequate field emergence. The most rapid method of selecting pods with immature viable seeds was to harvest pods that had begun to turn yellow. Pod yellowing occurred about 38 DAF of cultivars.

INTRODUCTION

The harvest of immature viable seeds has been suggested as a possibility for reducing generation time in soybean [*Glycine max* (L.) Merr.] breeding programs (8). Premature harvest could be useful for breeding

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programs in tropical nurseries for generation advance (8). In these environments, soybeans of Maturity Groups 0 to IV mature in approximately 90 days, and two generations can be grown during the 6-month period when they are not planted in their area of adaptation. By reducing the length of the generation time, it may be possible to obtain up to three generations in 6 months.

Several authors have reported a positive association between seed maturation, measured as days after flowering (DAF), and germination capacity of soybeans determined under laboratory conditions (1, 2, 4, 7, 11, 12, 13). In these studies cultivars were planted in their area of adaptation, and pods were harvested at various intervals at and after 22 DAF. The authors reported that immature viable seeds were obtained from air-dried pods pulled from plants about 30 DAF and later (1, 2, 4, 7, 11, 12, 13). Improved germination was observed when pods remained attached to the plants during air drying (13). There is no published information on field emergence of immature seeds of soybean genotypes adapted to temperate climates when grown in tropical environments. The first objective of this study was to determine field emergence of immature seeds of soybean genotypes adapted to temperate climates when they are grown in tropical environments.

Seed development determined as number of days after flowering requires tagging of individual flowers (2, 4, 12). This procedure, used for basic studies where exact measurements of seed maturation are needed, is inefficient for use in a practical breeding situation. Some visual indicator of when immature seeds are viable is needed to harvest pods from a large number of plants. Pod width (PW) has been used as an indicator of seed weight in soybeans (3, 10). The seed width (SW) to PW ratio has been used to determine when pods have reached maximum size (5). Pod color has served as a visual indicator of physiological maturity in soybean (6, 9, 14). A second objective of the study was to evaluate whether pod color or the ratios of SW to PW and SW to pod thickness (PT) could be used to determine when to harvest immature viable seed.

MATERIALS AND METHODS

The two cultivars used for the study were BSR 201 of Maturity Group II and Cumberland of Maturity Group III. They were planted in the Iowa State University Soybean Nursery at the Isabela Research Center of the University of Puerto Rico, Puerto Rico, in 1981 and 1982. Plantings on 18 November 1981 and 8 February 1982 were exposed to the natural daylength conditions of Puerto Rico, referred to as the unlighted (UL) environments. A second planting on 18 November 1981, referred to as the lighted (L) environment, was exposed to continuous light for 15 days after emergence, 14.5-hour days for an additional 35 days, and natural day length thereafter. Lighting consisted of 240-V, 1,500-W quartz-iodide bulbs installed on poles approximately 7 m high.

In the three plantings, each cultivar was grown in eight rows 18 m long and 0.60 m apart with 26 seeds per meter of row. The six center rows were used to harvest the material for the study. At flowering time, a tag was hung immediately below nodes with freshly opened flowers. The number of flowers per node and the flowering date were recorded on each tag. Old flowers and young buds were removed from the nodes upon tagging, and the nodes were checked twice subsequently to remove newly opened flowers. Harvest began 24 DAF and continued at weekly intervals until 59 DAF, when plants reached stage R8 (9). Two harvest procedures were used at every harvest date. Individual pods were pulled from plants to obtain a total of 250 seeds, and whole plants were pulled to obtain a total of 200 seeds. The 50 extra seeds obtained of pods pulled from plants on the date of harvest were used to determine fresh weight and were dried to constant weight to measure dry weight. Seed weights were recorded to the nearest 1 mg. Detached pods and those attached to plants were air-dried at ambient conditions for 3 weeks before they were hulled by hand.

Immediately after harvest, pod width (PW), pod thickness (PT), and seed width (SW) were recorded to the nearest 0.1 mm with a sample of 50 pods. PW was measured at the widest part of the terminal seed cavity as the distance from suture to suture (5). PT was measured perpendicular to the width at the terminal seed cavity as the distance from wall to wall (10). SW of the seed in the pod was measured from the suture of the terminal seed cavity at the point of seed attachment to the contour of the seed. The outline of the seed in the pod was visible by holding a flashlight with an aperture similar to that of the seed cavity under the pod. The ratio of SW to PW and SW to PT was expressed in percentage as $SW/PW = (SW \div PW) \times 100$ and $SW/PT = (SW \div PT) \times 100$. Pod color was recorded at each harvest date.

The 200 seeds obtained for each cultivar, harvest date, and harvest procedure were randomly divided in four lots of 50 seeds each. Three lots were used to plant a replicated test in the field. Field emergence was evaluated in a Coto soil (Typic Haplorthox, clayey, kaolinitic, isohyperthermic). A split-plot arrangement of a randomized complete block-design with three replications was used. Whole plots were the factorial combination of the three harvest environments × two cultivars. Subplots were the factorial combination of six harvest dates and a control × two harvest procedures. The control was a 50-seed sample from the original lot used to plant the three environments in Puerto Rico. Plots were single rows 2 m long, spaced 0.60 m between rows, and planted with 25 seeds per meter of row. Field emergence was determined by counting the number of plants per plot 20 days after emergence and expressed in percentage.

A germination test with two replications was conducted under laboratory conditions. There were no significant differences in field emergence between harvest procedures; therefore, the two 50-seed samples from each harvest procedure were used as replications. Seeds were placed on top of cellulose cloth on aluminum trays and watered with distilled water. After 5 days at 27 C and 90% relative humidity, the germination percentage was determined. A seed was considered germinated when the radicle pierced the seed coat and grew to twice the length of the cotyledons. Before the germination test, seed quality scores were assigned to every sample on the basis of percentage of seeds showing fungal and insect damage according to the scale: 1 = < 2%, 2 = 3%, 3 = 3 to 5%, 4 = 5 to 8%, 5 = > 8%. The percentage of immature seed, determined as those with a triangular shape or with green seed coat, also was recorded.

Analyses of variance were calculated. Environments and genotypes were considered random effects, and harvest dates and harvest procedures were fixed effects.

RESULTS AND DISCUSSION

Significant differences in field emergence were observed among harvest environments (table 1). The November L environment had the highest field emergence, 92%; the lowest percentage of immature seeds, 13%; and intermediate seed quality scores, 3.2 (tables 1 and 2). The February environment had the lowest field emergence percentage, 40%; the highest percentage of immature seed, 22%; and the poorest seed quality scores, 4.6. There were significant cultivar differences in the average field emergence across harvest environments: 80% for BSR 201, and 62% for Cumberland. At every harvest environment, the average field emergence of BSR 201 was higher than that of Cumberland. The difference between the two cultivars was particularly large in the February UL environment. In that environment, a heavy infestation by insects affected seed quality, which, in turn, caused reduced field emergence (tables 1 and 2). The prevalent insect, lima bean borer (*Etiella zinckenella*), fed directly on developing seeds.

There were no significant differences in field emergence between harvest procedures (data not shown). The similarity in field emergence for seeds obtained from pods pulled from plants and pods that remained attached to plants during the drying period suggested that the process of seed maturation is independent of the parent plant, as previously indicated (2).

There were significant differences among harvest dates in field emergence. Seeds obtained from pods harvested as early as 24 DAF had the capacity to emerge under field conditions, but the percentage was lower

TABLE 1.—*Average field emergence and germination percentages of seed of two cultivars harvested from 24 to 59 days after flowering in three environments*

Days after flowering	Harvest environment						Average
	November				February		
	Unlighted		Lighted		Unlighted		
	BSR 201	Cumberland	BSR 201	Cumberland	BSR 201	Cumberland	
				%			
				Field emergence			
24	30	34	90	84	38	4	47
31	90	81	93	86	77	20	74
38	94	88	92	94	78	26	79
45	94	92	95	96	68	23	79
52	92	87	94	92	61	15	74
59	92	86	92	92	65	6	72
x̄	82	78	93	91	64	16	71
LSD (0.05)[1]	15		13		12		5
				Germination			
24	15	51	77	96	28	11	46
31	42	90	93	97	55	44	70
38	95	99	99	100	80	43	86
45	92	98	95	100	42	35	77
52	86	99	97	98	42	28	75
59	88	99	100	100	52	19	76
x̄	70	89	94	98	50	30	72
LSD (0.05)	38		3		29		9

[1] LSD is the least significant difference with which to compare consecutive field emergence and germination percentages.

TABLE 2.—*Average seed quality scores, percentage of immature seeds, and pod color of two replications of seed of two cultivars harvested from 24 to 59 days after flowering in three environments*

Days after flowering	Seed quality[1]			Immature seeds			Pod color
	November		February	November		February	
	Unlighted	Lighted	Unlighted	Unlighted	Lighted	Unlighted	
		Score			%		
24	1.0	1.0	4.0	98	73	94	Green
31	1.0	3.0	4.5	17	5	12	Green
38	1.5	4.0	4.0	0	0	20	Green-yellow
45	2.0	2.5	5.0	0	0	2	Yellow
							Yellow-brown
52	1.5	4.0	5.0	0	0	1	Brown
59	1.0	4.5	5.0	0	0	0	Brown
x̄	1.3	3.2	4.6	19	13	22	
$S_{\bar{x}}$	0.2	0.4	0.2	8	6	7	

[1] Scores ranged from 1 (excellent) to 5 (poor).

TABLE 3.—*Average moisture percentage and fresh and dry weights of seed of two cultivars harvested from 24 to 59 days after flowering in three environments*

Days after flowering	Moisture	Weight	
		Fresh	Dry
	g kg⁻¹	*mg/seed*	
24	730	236	6
31	670	332	11
38	630	394	144
45	400	314	160
52	210	222	162
59	220	232	162
$S_{\bar{x}}$	41	20	10

TABLE 4.—*Average ratio of seed width to pod width, average ratio of seed width to pod thickness, and seed width of two cultivars harvested from 24 to 59 days after flowering in three environments*

Days after flowering	Harvest environments						$\bar{x}$	$\bar{x}$ Seed width
	November				February			
	Unlighted		Lighted		Unlighted			
	BSR 201	Cumberland	BSR 201	Cumberland	BSR 201	Cumberland		
				%				*mm*
			Seed width/pod width					
24	72	80	78	79	71	64	74	7.2
31	78	80	82	85	78	76	80	7.7
38	81	87	84	84	80	83	83	8.1
45	74	83	72	79	66	78	75	7.2
52	74	77	72	72	65	74	72	6.8
59	76	77	70	72	64	68	71	6.7
			Seed width/pod thickness					
24	130	134	116	128	120	131	126	
31	108	115	104	120	128	124	116	
38	108	118	106	116	112	127	114	
45	107	115	100	112	106	113	109	
52	98	112	98	110	106	117	107	
59	99	106	94	102	100	113	102	

than for other dates (table 1). For both cultivars at the three harvest environments, the largest increase in field emergence was observed between 24 and 31 DAF. Field emergence of seeds harvested at 31 DAF was significantly higher than that of seeds harvested at 24 DAF for both cultivars at the two UL environments. In the L environment, field emergence of seeds harvested 24 DAF was high and not significantly different from those harvested at 31 DAF. The laboratory germination test agreed with the observations of the field emergence test (table 1).

Maximum dry weight occurred at a later date that maximum fresh weight (table 3). Dry weight of seeds increased until 52 DAF, and fresh weight until 38 DAF. The largest gain in fresh weight occurred during the period from 24 to 31 DAF; in dry weight, from 31 to 38 DAF. At 31 DAF, seeds had accumulated 7% of their maximum dry weight and had a moisture content of 670 g/kg. Moisture content decreased from 24 DAF until 52 DAF, and maximum moisture loss occurred from 38 to 45 DAF. Moisture content and dry weight remained unchanged between 52 and 59 DAF.

SW increased from 24 to 38 DAF (table 4). Maximum SW occurred approximately 2 weeks before maximum dry weight (tables 3 and 4). From 38 DAF and thereafter, SW measurements decreased, suggesting that the process of seed maturation had started (2). The width of the seed continued to decrease after maximum dry weight was attained at 52 DAF. These data suggest that SW is dependent on fresh weight.

The SW/PW ratio combined over cultivars and environments was 74% at 24 DAF, reached a maximum value of 83% at 38 DAF, and decreased at later harvest dates (table 4). The SW/PW ratio did not reach 100% because the thickness of the suture on the side of the pod toward which the seed developed was not included in the SW measurement. At every harvest date, variable SW/PW ratios were observed across cultivars and environments. For example, at 24 DAF, the range was from 64 to 80%; at 31 DAF, from 76 to 85%; and at 38 DAF, from 80 to 87%.

SW/PT ratio combined over cultivars and environments was 126% at 24 DAF, and decreased until 59 DAF (table 4). At every harvest date, SW/PT ratios varied across cultivars and environments. For example, at 24 DAF, the range was from 116 to 134%; at 31 DAF, from 104 to 128%; and at 38 DAF, from 106 to 127%.

Pods harvested at 24 and 31 DAF were green (table 2). At 38 DAF they were turning yellow, and at 52 DAF they had already reached their mature pod color.

The results indicated that for temperate soybean genotypes grown in tropical conditions it is feasible to harvest immature seed that will have satisfactory field emergence. Immature seeds can be obtained from pods pulled from plants as early as 31 DAF and dried intact at ambient conditions. Seeds obtained at that harvest date had not reached maximum fresh and dry weights or maximum seed width. The percentage of triangular-shaped or green seeds at 31 DAF ranged between 5 and 17%.

Of the three indicators evaluated, pod color was the most reliable method of selecting pods with immature viable seeds. Pods harvested at approximately 38 DAF had begun to yellow and had accumulated 89% of their maximum dry weight. The SW/PW and SW/PT ratios were too variable and would not provide an objective method of determining when to harvest immature viable seed. The harvest of immature viable seeds at the earliest possible date, 31 DAF, would require the use of indicators other than pod color. Research is in progress to evaluate alternative indicators.

RESUMEN

Se investigó la cosecha de semillas inmaturas de soja [*Glycine max* (L.) Merr.] como una forma de acelerar el avance de generaciones en programas de fitomejoramiento. Un objetivo del experimento fue determinar la emergencia en el campo de semillas inmaturas consechadas de genotipos adaptados a climas templados cuando éstos se siembran en ambientes tropicales. El segundo objectivo fue comparar el color de la vaina y la relación del ancho de la semilla (SW) con el ancho de la vaina (PW) y SW al grosor de la vaina (PT) como indicadores de cuándo cosechar semillas inmaturas viables. Se sembraron dos cultivares en tres ambientes en la Iowa State University Soybean Nursery localizada en el Centro de Investigaciones y Desarrollo Agrícola de Isabela de la Universidad de Puerto Rico. Se comenzó a cosechar semillas 24 días después de la floración (DAF) y se continuó a intervalos semanales hasta los 59 DAF. Se usaron dos procedimientos para cosechar; en uno las vainas se arrancaron de las plantas y en el otro se cosecharon las plantas con vainas. Para cada procedimiento, fecha de cosecha, cultivar y ambiente se midieron la emergencia en el campo, PT, SW y AV, y se observó el color de las vainas. No se detectaron diferencias significativas en la emergencia en el campo entre los dos procedimientos para cosechar. Se observaron diferencias significativas en la emergencia en el campo de las semillas cosechadas entre los distintos ambientes, cultivares y fechas de cosecha. La emergencia en el campo observada con semillas cosechadas 31 DAF fue adecuada. El método mas rápido de seleccionar vainas con semillas inmaturas viables fue el de cosechar vainas que ya habían comenzado a amarillear. El cambio de color de las vainas, de verde a amarillo, ocurrió aproximadamente a los 38 DAF.

LITERATURE CITED

1. Adams, C. A., M. C. Fjerstad and R. W. Rinne, 1983. Characteristics of soybean seed maturation: necessity for slow dehydration, Crop Sci. 23: 265–67.
2. —— and R. W. Rinne, 1981. Seed maturation in soybeans [*Glycine max* (L.) Merr.] is independent of seed mass and of the parent plant, yet is necessary for production of viable seeds, J. Exp. Bot. 32: 615–20.
3. Bravo, J. A., W. R. Fehr and S. Rodríguez de Cianzio, 1980. Use of pod width for indirect selection of seed weight in soybeans, Crop Sci. 20: 507–10.
4. Burris, J. S., 1973. Effect of seed maturation and plant population on soybean seed quality, Agron. J. 65: 440–41.
5. Cianzio, S. Rodríguez de, S. J. Frank and W. R. Fehr, 1982. Seed width to pod width

ratio for identification of green soybean pods that have attained maximum length and width, Crop Sci. 22: 463–66.

6. Crookston, R. K. and D. S. Hill, 1978. A visual indicator of the physiological maturity of soybean seed, Crop Sci. 18: 867–70.
7. Dharmasena, C. D., 1981. Soybean seed maturation at different levels in the plant canopy and viability of seed harvested at different stages, Int. Agric. Publ. INTSOY Ser. 22: 11–18.
8. Fehr, W. R., 1976. Description and evaluation of possible new breeding methods for soybeans, p. 268–75. In L. D. Hill (Ed). World Soybean Research, Interstate Printers and Publishers, Inc., Danville, Ill.
9. —— and C. E. Caviness, 1977. Stages of soybean development, Iowa Agric. Home Econ. Exp. Stn. Special Rep. 80.
10. Frank, S. J. and W. R. Fehr, 1981. Associations among pod dimensions and seed weight in soybeans, Crop Sci. 21: 547–50.
11. Green, D. E., 1961. A study of factors affecting soybean seed quality, Unpublished M.S. Thesis. Univ. Missouri, Columbia, MO.
12. Obendorf, R. L., E. N. Ashworth and G. T. Rytko, 1980. Influence of seed maturation on germinability in soybean, Crop Sci. 20: 483–86.
13. Ozaki, K., M. Saito and K. Nitta, 1956. Studies on the seed development and germination of soybean plants at various ripening stages, Hokkaido Natl. Exp. Stn. Res. Bull. 70: 6–14.
14. Tekrony, D. M., D. B. Egli, J. Balles, T. Pfeiffer and R. J. Fellows, 1979. Physiological maturity in soybean, Agron. J. 71: 771–75.