A visual indicator for harvest of immature viable seed of indeterminate soybean genotypes[1]

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ABSTRACT

Harvest of immature soybean [*Glycine max* (L.) Merr.] seed is used in breeding nurseries to reduce generation length. Individual pods are harvested and usually dried intact at ambient conditions. Number of days after flowering (DAF) has been shown to be a good criterion to determine the appropriate time to harvest, but the method is time consuming on a large number of genotypes. The objective of this study was to identify a visual indicator of the appropriate stage for harvest of immature viable seeds. Two indeterminate cultivars, Lakota from Maturity Group (MG) I and BSR 301 from MG III, were planted in three environments at Isabela, PR. Harvest of pods began 28 DAF and continued every 3 days until 46 DAF. Field emergence, fresh and dry seed weights, seed width, seed length, seed thickness, pod angle, pod color and reproductive stage of individual plants were recorded. Results pointed out that the best visual indicator for the harvest of immature seed obtained from green pods was a combination of pod angle, seed dimensions, and reproductive stage of the plant. In general, field emergences of 70% or better were obtained with immature seed of green pods that could be bent no more than 30° before rupturing and that contained seeds at or near their maximum width, length, and thickness. These pods were harvested from plants that had reached reproductive stage R6.

RESUMEN

Indicador visual para la cosecha de semillas inmaturas viables en genotipos indeterminados de soja

La cosecha de semillas inmaturas de soja, *Glycine max* (L.) Merr., se utiliza en los semilleros de fitomejoramiento para acortar la longitud de las generaciones. Generalmente se cosechan vainas individuales que se secan intactas en el ambiente. Un procedimiento apropiado para determinar el momento de cosecha de las vainas es anotar los días después de la floración (DAF). El método de marcar las flores consume, sin embargo, mucho tiempo, sobre todo cuando se trabaja con un gran número de genotipos. El objetivo de este estudio fue identificar un indicador visual para determinar el momento más apropiado de la cosecha de semillas

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inmaturas viables. Se usaron dos cultivares indeterminados, Lakota del Grupo de Madurez (MG) I y BSR 301 del MG III, que se sembraron en tres ambientes en Isabela, PR. La cosecha de las vainas comenzó a los 28 DAF y continuó cada 3 días hasta 46 DAF. Se tomaron los siguientes datos: germinación en el campo; pesos húmedo y seco de las semillas; ancho, longitud y grosor de las semillas; ángulo de la vaina; color de la vaina y etapa reproductiva de las plantas individuales. Los resultados indicaron que el mejor indicador visual para la cosecha de semillas inmaturas de vainas verdes fue la combinación del ángulo de la vaina, las dimensiones de las semillas y la etapa reproductiva de las plantas. En general, la germinación fue de 70% y se obtuvieron mejores germinaciones con semillas inmaturas de vainas verdes que tenían un angulo de flexión no mayor a los 30° sin que se provocara su rotura y que contenían semillas con las dimensiones máximas, o próximas a ellas, en cuanto a su ancho, longitud y grosor. Estas vainas se cosecharon de plantas individuales que estaban en la etapa reproductiva de R6.

INTRODUCTION

The harvest of immature seed in soybean, *Glycine max* (L.) Merr., breeding programs can reduce the length of a generation. Ortiz et al. (7, 8) harvested immature seeds of indeterminate soybean genotypes in tropical environments reducing the length of a generation by approximately 10 to 14 days. This savings in time is of importance in obtaining a maximum number of generations each year when the crop is alternatively planted at its adapted region and in an unadapted location in off-season plantings (1).

For efficient harvest of immature seed, visual indicators are needed to determine harvest date. Visual indicators of physiological maturity for individual soybean seed and for the whole plant have been determined (4, 5, 6, 9, 10). There is no published information, however, of an appropriate visual indicator for the harvest of immature viable soybean seed. Ortiz et al. (7) evaluated three visual indicators: pod color, the ratio of seed width (SW) to pod width, and the ratio of SW to pod thickness. They concluded that pod color was the most reliable of the three visual indicators. For indeterminate genotypes of Maturity Groups III and earlier grown in tropical locations, pods harvested approximately 38 days after flowering (DAF) had begun to turn yellow and seeds had accumulated 89% of their maximum dry weight. The average field emergence of the seed was 79%.

One limitation to the use of pod color as a visual indicator is that immature viable seeds with average field emergence of 74% (not significantly different from the previously report of 79%) can be harvested before any yellowing of the pods occurs (7). DAF is not a practical indicator of time of harvest because recording flowering dates on a large number of genotypes is a time-consuming task. A visual indicator of the appropriate stage for the harvest of green pods containing immature viable seeds is needed. The objective of our study was to evaluate pod and seed characteristics in relation to pods, as visual indicators for harvesting viable immature soybean seed.

MATERIALS AND METHODS

Two indeterminate cultivars, Lakota of Maturity Group (MG) I and BSR 301 of MG III, were planted in three environments at the Iowa State University-University of Puerto Rico nursery near Isabela, Puerto Rico, during 1983 and 1984. The Puerto Rico environments are located at 18° 30″ N, 67° 00″ W. Plantings on 1 November 1983 and 1 February 1984 were exposed to natural day length and are referred to as unlighted environments (UL). The third planting on 1 November 1983, referred to as the lighted environment (L), was exposed to continuous light for 15 days after emergence, 14.5-h days for an additional 35 days, and natural day length thereafter. Lighting consisted of four 240-V, 1500-W quartz-iodide bulbs installed on a pole approximately 7 m high. The light intensity from this source is sufficient to extend day length, delaying flowering by approximately 10 days compared to that of plantings with natural day length conditions of Puerto Rico (2).

For each of the three plantings, cultivars were grown in a six-row plot 18 m long with a row spacing of 0.60 m and at a planting rate of 26 seeds per meter in the row. The soil belongs to the Coto series (clayey, kaolinitic, isohyperthermic Typic Haplorthox). Plots were irrigated with a sprinkler system as often as needed during the growing season. The four center rows were used for harvest of pods and seed for the study.

Seed maturation was measured as days after flowering (DAF). The procedure used for estimating age of seed at harvest was described by Ortiz et al. (7). At flowering, a tag was attached immediately below a node on the main stem with freshly opened flowers. Old flowers and young buds were removed from the node. Flowering date and number of flowers per node were recorded on the tag. Tagged nodes were examined approximately 2 weeks later to remove any newly opened flowers. To obtain sufficient seed of similar physiological age, tagging was done on 3 consecutive days.

Harvest of pods began 28 DAF and continued at 3-day intervals until 46 DAF. At each harvest date, individual pods were pulled from as many plants as possible to obtain a total of 200 seeds. The number of pods to harvest was calculated assuming an average of two seeds per pod. Reproductive stage of the plants was determined at each date of harvest (5).

Pod color, pod flexibility, and seed dimensions were recorded on a random sample of 25 pods immediately after harvest. Pod flexibility was the angle formed when the two ends of the pod were bent toward each other as much as possible without rupturing (fig. 1). The angle was meas-

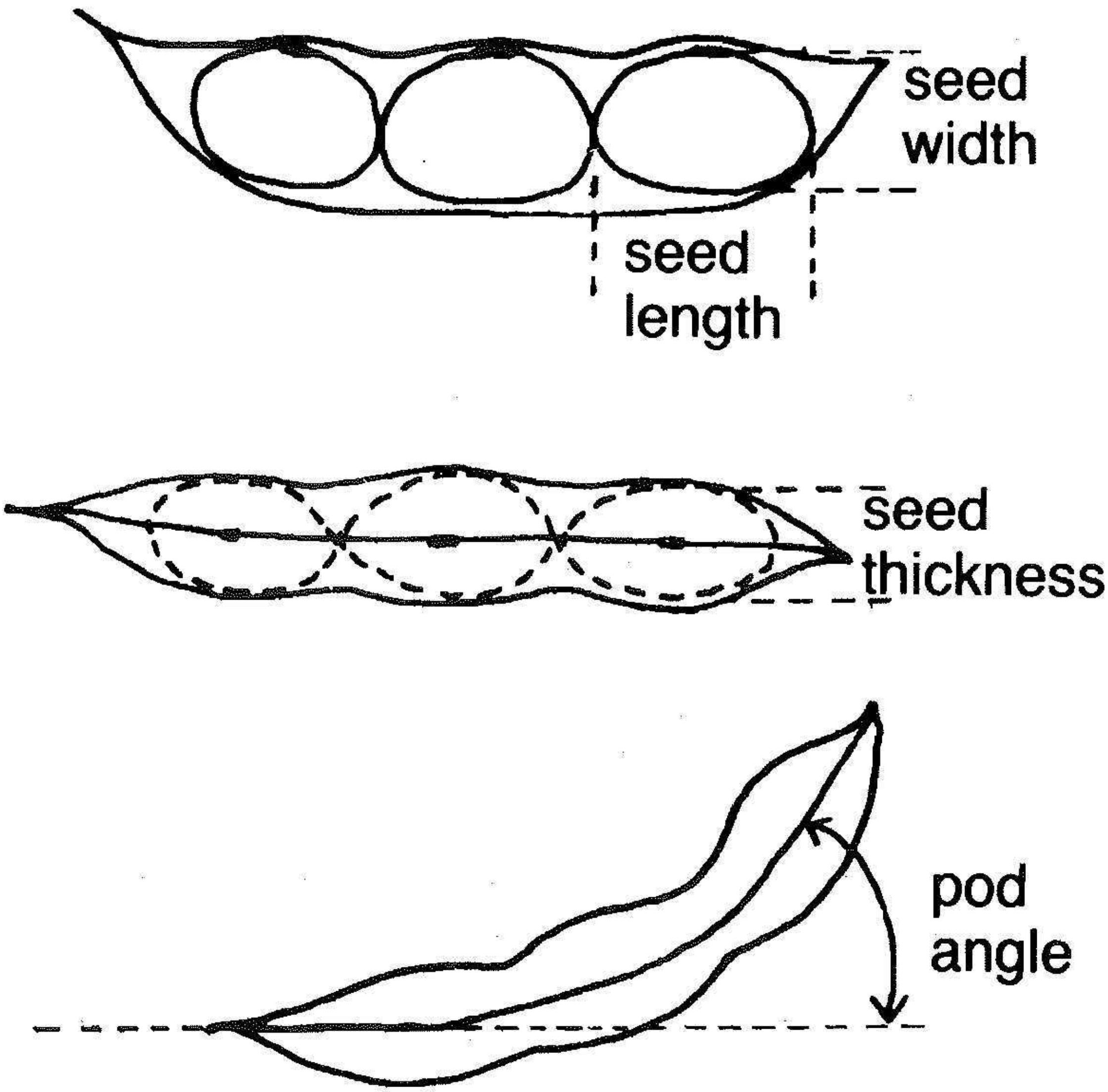

FIG. 1.—Pod and seed dimensions measured in the study.

ured by placing the straight side of a protactor along the suture of the pod and bending the pod as far as possible without rupturing.

Dimensions of the seed in the terminal seed cavity were recorded with a caliper to the nearest 0.1 mm (fig. 1). The outline of the seed in the pod was enhanced by holding a flashlight under the pod with an aperture similar to that of the seed cavity. Seed width (SW) was measured as the width of the seed from suture to suture at the point of seed attachment (3). Seed length (SL) was measured parallel to the pod suture, and seed thickness (ST) was measured perpendicular to the dimension for SW. Fresh weight was determined from a random sample of 50 seed. The same sample was dried to constant weight to determine dry weight. Seed weights were recorded to the nearest 1 mg.

Pods were air-dried outdoors for 3 weeks before being shelled by hand. Pods harvested at each date were placed in mesh bags approximately 30 cm long and 15 cm wide. Bags were hung from a wire suspended approximately 2 m from the ground in an aerated location and exposed to sunlight from 7 am to 4 pm. At night and on rainy days, the bags were maintained in an air-conditioned seed laboratory. For pods harvested from the November planting, the mean relative humidity during the drying period was 81%, and the mean temperature was 23°C. For the harvest of the February planting, the mean relative humidity was 74%, and the mean temperature was 25°C.

Field emergence percentage was evaluated in three replications of a split-plot arrangement of a randomized complete-block design. Whole plots were the factorial combination of the three harvest environments and the two cultivars. Subplots were planted with seed harvested at each of the seven harvest dates in the three environments. Each plot was planted with 50 seeds in single rows 2 m long with a row spacing of 60 cm between rows. Seed was planted 5 cm deep in a Coto soil (Typic Haplorthox, clayey, kaolinitic, isohyperthermic) at the Isabela station in June 1984. Field emergence percentage was determined by counting the number of plants per plot 24 days after planting. In field plantings in Puerto Rico, field emergence of mature seed occurs approximately 4 days after planting (Cianzio, personal observations). For analyses of variance, environment was considered a random effect, and genotypes and harvest dates were considered fixed effects.

RESULTS AND DISCUSSION

Significant differences in field emergence were observed among environments [$LSD_{(0.01)} = 5$] and harvest dates [$LSD_{(0.01)} = 8$] (table 1). The average field emergence of the cultivar Lakota was 76%, significantly [$LSD_{(0.01)} = 4$] different from the average field emergence of BSR 301, which was 60% (data not shown). The November UL had the greatest average field emergence and February UL the least (table 1). Throughout the duration of the study, insect populations were controlled by weekly spraying of insecticide. In the February UL environment, however, seed damage by insects, which may have affected field emergence was observed. Similar results also were reported by Ortiz et al. (7, 8). In the February plantings in Puerto Rico, seed development occurs in April and May, the onset of the rainy period and when natural photoperiod lengthens, two factors that are important in determining the levels of natural insect populations (8).

More than one standard can be utilized to determine the earliest stage of pod and seed development at which an acceptable field emergence percentage has been achieved. One criterion to determine an acceptable

TABLE 1.—*Mean field emergence percentage and percentage of immature seeds of two cultivars harvested from 28 to 46 days after flowering in three environments.*

	Harvest environment						
	November				February		
	Unlighted		Lighted		Unlighted		
Days after flowering	Lakota	BSR 301	Lakota	BSR 301	Lakota	BSR 301	$\bar{x}$
	%						
	Field emergence						
28	61	63	60	0	59	19	44
31	73	68	71	8	57	37	52
34	80	77	91	48	79	61	73
37	75	70	78	72	78	67	73
40	81	77	87	79	80	64	78
43	98	71	83	81	73	81	81
46	99	62	91	94	54	57	76
$LSD_{(0.01)}$[1]	14	16	13	18	16	11	
$\bar{x}$	81	70	80	54	68	55	68
$LSD_{(0.01)}$[2]	14		14		13		
$\bar{x}$	76		67		62		
$LSD_{(0.01)}$[3]			5				8

[1]Least significant difference with which to compare field emergence of harvest dates of each cultivar at each environment.

[2]Least significant difference with which to compare field emergence of harvest dates at each environment.

[3]Least significant difference with which to compare field emergence of environments and harvest dates.

field emergence could be a statistical comparison between the stage at which the largest emergence percentage was obtained and the emergence percentage at another stage. In this study, an acceptable field emergence was determined by computing the least significant difference (LSD) at the 0.01 probability level for each cultivar and environment (table 1). Any stage not differing by more than the LSD from the highest field emergence percentage obtained for a cultivar in an environment was considered acceptable.

Substantial differences were observed between cultivars and among environments for the number of DAF before an acceptable field emergence was obtained according to the criterion described (table 1). Acceptable field emergence was achieved as early as 28 DAF for BSR 301 in the November UL environment, and as late as 43 DAF for Lakota in the same environment. In the November L environment, acceptable field emergences were obtained at 37 DAF for Lakota and at 40 DAF for BSR 301. In the February UL environment, acceptable field emergences were obtained at 34 DAF for Lakota and at 37 DAF for BSR 301. The average field emergence percentage for all environments and cultivars obtained using this criterion was 77%, ranging from 63 to 98% among environments and cultivars.

In a breeding program, acceptable field emergence would be established by the breeder as the maximum reduction in emergence percentage that could be tolerated with immature seeds relative to the percentage that could be obtained with mature seeds. On the basis of previous observations (Cianzio, personal communication), a 70% average field emergence for immature seed is considered an appropriate estimate for practical breeding purposes.

In general, field emergences of 70% were observed at the three environments obtained with immature seed harvested at 31, 34, or 37 DAF, except for BSR 301 in the February UL environment (table 1). In this case the field emergence observed at 37 DAF was 67% and at 43 DAF the field emergence was 81%.

The three seed dimensions of each cultivar recorded at each harvest date and environment followed a similar growth pattern (table 2). In the November UL environment, SW, SL, and ST of Lakota increased until 40 DAF, decreasing at 43 DAF and later dates. For BSR 301, maximum seed dimensions were observed at 43 DAF. In the November L environment, maximum seed dimensions for the two cultivars were observed at 43 and at 46 DAF. In the February UL environment, maximum seed dimensions for Lakota were observed at an earlier date compared to the November environments, 31 and 34 DAF. For BSR 301, the maximum dimensions were observed at 40 DAF. Maximum fresh weights also occurred at the same harvest dates in which maximum seed dimensions were observed (table 3), indicating that the process of seed drying had begun. An increase in seed dry weight was observed from 28 DAF until 46 DAF.

Pod angle (table 4) followed a pattern of behavior similar to seed dimensions (table 2) and seed fresh weight (table 3). In each environment pod angle of both cultivars decreased with the increase of SW, SL, ST and fresh weight. As seed approached maximum dimensions in the pod, the pod became less flexible as indicated by a decreased pod angle. Visual seed observation of green pods harvested at each harvest date and the corresponding seed dimensions indicated that soybean seed first expands and then shrinks.

Plants were at reproductive stage R6 at the time of maximum seed dimensions (table 4). For both cultivars in each environment, pods were green when harvested at the time of maximum seed dimensions, except

TABLE 2.—*Mean seed width, seed length, and seed thickness of 25-pod samples of two cultivars harvested from 28 to 46 days after flowering in three environments.*

Days after flowering	Harvest environment: November, Unlighted: Lakota	November, Unlighted: BSR 301	November, Lighted: Lakota	November, Lighted: BSR 301	February, Unlighted: Lakota	February, Unlighted: BSR 301
	mm/seed					
	Seed width					
28	8.5	8.0	7.6	5.2	8.0	8.3
31	8.5	8.0	7.9	7.2	8.3	8.3
34	8.5	8.3	7.8	7.9	8.0	8.3
37	8.8	8.6	8.1	8.2	7.9	8.3
40	8.5	8.8	7.8	8.2	6.5	8.6
43	7.0	8.9	8.4	8.4	6.5	8.1
46	7.0	8.9	8.0	8.8	6.1	6.6
$\bar{x}$	8.1	8.5	7.9	7.7	7.3	8.1
$LSD_{(0.01)}$[1]	0.8	0.4	0.3	1.3	1.0	0.7
	Seed length					
28	11.4	10.9	10.1	6.9	11.0	11.3
31	11.6	10.6	10.7	9.4	11.4	11.4
34	12.0	11.9	10.8	10.5	11.3	11.4
37	12.1	12.0	11.3	11.3	11.1	11.2
40	11.8	12.7	11.1	11.4	8.0	11.7
43	8.6	12.9	11.4	11.7	7.0	11.0
46	7.6	12.9	11.5	12.0	7.0	7.3
$\bar{x}$	10.7	12.0	11.0	10.4	9.5	10.8
$LSD_{(0.01)}$[1]	1.9	1.0	0.5	1.9	2.2	1.6
	Seed thickness					
28	5.3	5.1	5.1	2.8	6.2	5.5
31	6.3	5.5	5.3	4.3	6.6	5.9
34	6.6	5.8	5.6	5.0	6.7	6.3
37	6.6	6.6	6.1	5.7	6.5	6.4
40	6.8	6.7	6.2	5.9	5.6	6.6
43	5.8	7.2	6.6	6.2	5.9	6.2
46	5.9	7.1	6.4	6.6	5.8	5.6
$\bar{x}$	6.2	6.3	5.9	5.2	6.2	6.1
$LSD_{(0.01)}$[1]	0.6	0.9	0.6	1.4	0.4	0.4

[1]Least significant difference with which to compare seed width, seed length and seed thickness of harvest dates.

TABLE 3.—*Mean fresh and dry seed weight of 50-seed samples of two cultivars harvested from 28 to 46 days after flowering in three environments.*

Days after flowering	Harvest environment					
	November				February	
	Unlighted		Lighted		Unlighted	
	Lakota	BSR 301	Lakota	BSR 301	Lakota	BSR 301
	mg/seed					
	Fresh seed weight					
28	286	246	2046	64	318	298
31	340	274	244	146	344	300
34	364	300	250	226	354	348
37	366	366	292	274	346	340
40	350	400	280	308	210	370
43	232	446	334	346	164	326
46	174	430	320	370	160	174
$\bar{x}$	302	352	275	248	271	308
$LSD_{(0.01)}$[1]	78	82	48	116	93	68
	Dry seed weight					
28	82	5	42	4	94	110
31	108	68	60	22	124	116
34	116	74	72	32	140	130
37	124	102	72	54	154	140
40	136	120	90	78	154	154
43	136	140	118	96	148	160
46	138	150	114	114	150	154
$\bar{x}$	120	94	81	57	138	138
$LSD_{(0.01)}$[1]	21	52	29	42	23	21

[1]Least significant difference with which to compare fresh and dry seed weights of harvest dates.

for Lakota in the February UL environment, which had green-yellow pods.

Our results point out that the best visual indicator for the harvest of immature seed obtained from green pods was a combination of pod angle, seed dimensions, and reproductive stage of the plant. In general, field emergences of 70% or better were obtained with immature seed of green pods that could be bent no more than 30° before rupturing and contained seeds at or near their maximum length, width, and thickness. These pods were harvested from plants that had reached reproductive stage R6.

TABLE 4.—*Mean pod angle and pod color of a 25-pod sample and reproductive stages of plants of two cultivars harvested from 28 to 46 d after flowering in three environments.*

Days after flowering	Harvest environment: November, Unlighted, Lakota	November, Unlighted, BSR 301	November, Lighted, Lakota	November, Lighted, BSR 301	February, Unlighted, Lakota	February, Unlighted, BSR 301
			Pod angle			
28	74	100	100	151	24	43
31	70	84	88	133	13	31
34	27	64	70	115	17	14
37	14	21	34	88	22	20
40	19	25	27	78	91	16
43	97	16	19	49	108	55
46	112	18	30	20	113	105
$\bar{x}$	59	47	52	91	56	41
$LSD_{(0.01)}$[1]	41	37	34	48	48	34
			Pod color[2]			
28, 31, 34	Gr	Gr	Gr	Gr	Gr	Gr
37	Gr	Gr	Gr	Gr	Gr-Y	Gr
40	Gr-Y	Gr	Gr	Gr	Y-Br	Gr
43	Y-Br	Gr	Gr-Y	Ge	Br	Gr-Y
46	Br	Gr	Gr-Y	Gr	Br	Br
			Reproductive stage of plants			
28	R5-R6	R5	R5	R5	R5-R6	R5
31	R5-R6	R5	R5	R5	R6	R5
34	R6	R5-R6	R5-R6	R5	R6	R6
37	R6	R6	R6	R5	R6	R6
40	R6	R6	R6	R5-R6	R6-R7	R6
43	R7	R6	R6	R6	R8	R7
46	R8	R6	R6	R6	R8	R8

[1]Least significant difference with which to compare pod angle of harvest dates.
[2]Gr = green, Y = yellow, Br = brown.

Plants from which appropriate green pods could be harvested were at R6. At that stage, pods of indeterminate cultivars at the bottom of the plant could be harvested because of their advanced development. For the harvest of immature viable seeds, plants can be evaluated rapidly at regular intervals for overall stage of development. Evaluation of pod flexibility and seed dimensions can be initiated when stage R6 is attained.

When harvesting a large number of green pods, such as from segregating populations, it is too time consuming to test each pod for flexibility. Pod angle can be tested on several pods to obtain a visual impression of seed dimensions associated with a pod angle of less than 30°. Pods bending more than 30° are not as thick or as round as more rigid pods. When visual relationships between pod flexibility and seed dimensions have been established, pods can be harvested on the basis of their overall size. If there is uncertainty about the suitability of an individual pod for harvest, its pod flexibility can be tested.

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