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Breeding for improved emergence of low-phytate soybean lines

By

Brian Paul Anderson

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

Master of Science

Major: Plant Breeding

Program of Study Committee:
Walter R. Fehr, Major Professor
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Iowa State University

Ames, Iowa

2007

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Acknowledgements

I would like to thank Dr. Fehr for giving me the opportunity to work and learn in the soybean breeding program. Many thanks to Grace Welke, Susan Johnson, and Kevin Scholbrock for their guidance and hard work. I would also like to thank my fellow graduate students Curtis Scherder, Jordan Spear, Raechel Baumgartner, Jonathan Jenkinson, and Loren Trimble for their continual input and feedback during my studies. Finally, I would like to thank my wife LeAnn for her patience, love, and support.

Abstract

Seed production in subtropical environments is commonly used by soybean [*Glycine max* (L.) Merr.] breeders to reduce the number of years required for cultivar development. One objective of this study was to determine if seed source would impact the field emergence of low-phytate (LP) lines with the *pha1* and *pha2* alleles. Seed of six BC_3F_4-derived LP lines, the LP donor parent CX1834-1-6 (CX1834), and the normal-phytate (NP) recurrent parent B01769B019 (B019), was harvested from the field at Ames, IA, in 2005 (IA), at Ponce, Puerto Rico, in January 2007 (PR-Jan), and at Ponce, in May 2007 (PR-May). The three seed sources of the eight lines were evaluated at three Iowa locations in 2007. The mean field emergence of LP lines was 77.6% for the IA source, 70.1% for the PR-Jan source, and 25.4% for the PR-May source while that of B019 ranged from 80.3 to 82.0% for the three sources. The seed source used to plant lines with the *pha1* and *pha2* alleles can have a significant influence on their field emergence, which can impact the development and evaluation of LP lines and on seed increases for commercial production.

A second objective of this study was to determine if the improved emergence of LP lines developed at Iowa State University would be inherited by their progeny when crossed with conventional NP cultivars to form single-cross populations or used as the donor parent of the LP trait to form backcross populations. Sixteen LP $F_{3:5}$ single-cross lines, 33 LP $BC_1F_{2:4}$ backcross lines, four LP parent lines, and six NP parent lines were evaluated for field emergence at three Iowa locations in 2007. The seed used to plant the test was harvested from the PR-May environment. The single-cross lines had a mean field emergence of 42.8% and a range of 6.9 to 69.8%, the

backcross lines had a mean of 31.4% and a range of 5.5 to 55.5%, the LP parent lines had a mean of 44.9% with a range of 30.1-59.0%, and the NP parent lines had a mean of 71.4% and a range of 46.1-80.8%. The results indicated that the LP lines with improved field emergence did not convey the trait to all of their progeny when evaluated with seed from PR-May.

Introduction

Phytate (*myo*-inositol 1,2,3,4,5,6-hex*akis*phosphate) is the major storage form of phosphorus (P) in normal-phytate (NP) soybean lines (Oltmans et al., 2005). Non-ruminant animals, such as swine and poultry, are unable to fully utilize the P in phytate due to their inability to produce sufficient phytase enzyme (Cromwell et al., 1995). Livestock producers add inorganic P and phytase enzyme to the rations of non-ruminants to meet their mineral requirements (Powers et al., 2006). Phytate also forms salts with divalent cations, such as zinc and iron, rendering them unavailable (Erdman and Poneros-Schneier, 1989). Both phytate P and bound cations are excreted in animal waste, potentially causing negative environmental impact (Daverede et al., 2004).

The four genetic sources of the LP trait reported in soybean are the *mips* allele, the combination of the *pha1* and *pha2* alleles, the *Gm-lpa*-TW-1 allele, and the *Gm-lpa*-ZC-2 allele (Sebastian et al., 2000; Oltmans et al., 2004; Yuan et al. 2007). The *mips*, *pha,* and *Gm-lpa*-TW-1 genetic sources of the trait can reduce the field emergence of soybean (Meis et al., 2003; Hulke et al., 2004; Oltmans et al., 2004, Yuan et al. 2007). By backcrossing the *pha1* and *pha2* alleles from the LP line CX1834-1-6 (CX1834) into the NP line B01769B019 (B019), some LP lines were recovered that had similar field emergence to the recurrent parent when planted with seed harvested in Puerto Rico during January 2005 (Spear and Fehr, 2007). When the LP lines with normal field emergence that they identified were planted at Ames, IA, in 2006 with seed harvested in Puerto Rico during May, all of the LP lines had significantly lower field emergence than the NP recurrent parent. This suggested that

the influence of seed source on the field emergence of LP lines with the *mips* and *Gm-lpa*-TW-1 alleles also may be a factor in the field emergence of LP lines with the *pha1* and *pha2* alleles (Meis et al., 2003; Yuan et al., 2007). One objective of this study was to determine if the source of seed used to plant LP lines with the *pha1* and *pha2* alleles can influence their field emergence.

The LP lines with normal field emergence developed by Spear and Fehr (2007) were crossed to NP lines to form single-cross populations and were used as the donor parents of the *pha* alleles to develop backcross populations using the NP parents as the recurrent parent. A second objective of this study was to determine if the improved emergence of LP lines observed by Spear and Fehr (2007) would be inherited by their progeny when crossed to NP lines for cultivar development.

Literature Review

Importance of the low-phytate trait

Phosphorus (P) is an essential element for animal growth and development. Phytate is the main storage form of P in soybeans, accounting for up to 85% of total P (Raboy et al., 1984). Soybean meal contains sufficient total P for the dietary needs of most non-ruminants, however, phytate P is mostly unavailable to non-ruminant animals. Only 25% of the P in soybean meal is available to swine (Cromwell et al., 2002). Phytate P is largely unavailable to non-ruminants because they do not produce sufficient amounts of the phytase enzyme, which is responsible for breaking down phytate to make the P available to the animal (Cromwell et al., 1993). Phytate also binds essential cations like zinc, calcium, and iron, rendering these elements unavailable as well (Erdman and Poneros-Schneier, 1989).

The phytate P that is not utilized by the animal is excreted, causing environmental concerns if managed improperly. P may be transported by surface runoff to streams and lakes and become a major cause of eutrophication, which leads to decreased water quality (Daverede et al., 2004). The diets of non-ruminant animals may be supplemented with phytase to help make the P more available to the animal and to decrease the total amount of P excreted. Soybean meal supplemented with phytase was shown to decrease the necessary dietary P intake by 25% and decrease excreted P by 31% (Cromwell et al., 1995).

Another method of increasing P availability is to decrease phytate P and increase inorganic P in soybean seed. Wilcox et al. (2000) used mutagenesis to isolate soybean mutants with reduced phytate and elevated inorganic P. The mutant

low-phytate (LP) lines had up to 70% of total P in the form of inorganic P, compared with 15% of total P in the form of inorganic P for normal- phytate (NP) cultivars.

LP soybean meal has been shown to be useful for increasing the availability of P to non-ruminant animals. Powers et al. (2006) studied the effects of feeding LP soybean meal to swine. They evaluated four rations of soybeans; NP meal without phytase (NP-np), NP meal with phytase added (NP-p), LP meal without phytase (LP-np), and LP meal with phytase added (LP-p). They found that LP-np soybean meal had a P digestibility of 50.3%, LP-p 47.6%, NP-np 43.7%, and NP-p 41.0%. The amount of water-soluble P (WSP) in fecal matter, an indicator of environmental impact, on a g WSP per kg feces basis was 8.5 for LP-p, 9.1 for LP-np, 10.1 for NP-p and 10.7 for NP-np. This indicated that there was a benefit in feeding LP soybean meal compared to NP soybean meal, even when phytase is added.

Inheritance of the low-phytate trait

The line with the LP trait used for this study was CX1834-1-6 (CX1834), which was developed by the USDA-ARS and Purdue University (Wilcox et al., 2000). They treated seeds of the breeding line CX1515-4 with ethyl methanesulfonate (EMS). M_3 seeds from each M_2 plant were screened for elevated inorganic P, a phenotype associated with reduced phytate. Two progeny, M153 and M766, were identified as LP mutants. M153 was crossed to the cultivar Athow and CX1834 was selected for its seed yield among the LP progeny from the cross.

Oltmans et al. (2004) made reciprocal crosses between CX1834 and A00-711013, a NP line developed by Iowa State University. All of the F_1 seeds had NP, which indicated complete dominance of the wild-type allele and no maternal effects.

F_2 seed was tested for the presence of the LP trait. Thirteen F_2 seeds were LP and 197 were NP, which satisfactorily fit a phenotypic ratio of 15:1. This ratio indicated that the low-phytate trait was controlled by two recessive alleles, designated *pha1* and *pha2*, at independent loci exhibiting duplicate dominant epistasis.

Walker et al. (2006) conducted research to identify molecular markers associated with the LP trait in CX1834-1-2, a sister line of CX1834. They crossed CX1834-1-2 to the line AGS Boggs-RR to develop segregating F_2 populations. The *pha1* allele was mapped to Satt237 (LG N) and the *pha2* allele was mapped to Satt561 (LG L). The *pha1* allele explained 40% of the variation in phytic acid content and the *pha2* allele explained 11% of the variation. Their data fit an additive model for the two alleles and supported the epistatic interaction of recessive alleles at two independent loci as reported by Oltmans et al. (2004).

Association of low phytate with agronomic and seed traits

Meis et al. (2003) evaluated seedling emergence and germination of LP lines containing the *mips* allele, a recessive allele that has a pleiotrophic effect on raffinose saccharides. They evaluated six LP *mips* lines and four NP lines for seedling emergence in the field and germination in three laboratory tests; warm germination, cold vigor, and accelerated aging. The NP lines had significantly better field emergence and germination in the accelerated aging test than the LP lines. They also noted a significant interaction between seed source and seedling emergence. Averaged across three Iowa environments, the LP *mips* lines grown from seed produced in the subtropical environments of Waimea, HI; Salinas, PR; and Puerto Vallarta, Mexico, had a field emergence of only 8%, whereas LP *mips* lines grown

from seed produced in the temperate environments of Bethany, MO; Atlantic, IA; and Semillas, Chile, and had a field emergence of 63%.

Hulke et al. (2004) evaluated a population that was developed by backcrossing the LP trait into a line low in saturated fatty acids. CX1834 was crossed to B01769B019 (B019), a low-saturate line developed jointly by Iowa State University and Pioneer Hi-Bred International, Inc. The low-saturate content was due to the recessive alleles *fap1* and *fap3* at two independent loci that have an additive effect on the reduction of palmitic acid content (Fehr et al., 1991; Schnebly et al., 1994). A backcross was made to B019 and $BC_1F_{2:3}$ seeds were tested for phytate content. Twenty LP and 20 NP $BC_1F_{2:4}$ lines with low-saturated fat content were evaluated at three Iowa locations. The LP lines had 22% lower mean field emergence than the NP lines. LP lines were not significantly different from NP lines for yield, maturity, lodging, height, protein, or oil. Some of the LP lines had greater emergence than the LP donor parent CX1834, which indicated that there was potential for selection of improved emergence in LP lines.

Oltmans et al. (2005) evaluated the agronomic and seed performance of LP and NP lines from three populations formed by crossing CX1834 to three NP cultivars: IA1008, IA2050, and IA2068. Ten LP and 10 NP $F_{2:4}$ lines from each population were grown at three Iowa locations in 2003. They found that the LP lines had an average of 23% lower field emergence than the NP lines across locations. There was a significant difference for field emergence among the LP lines in each of the populations, indicating the potential for breeding for higher emergence of LP lines.

Spear and Fehr (2007) identified LP lines selected from a population developed by additional backcrosses to B019 beyond that of the population studied by Hulke et al. (2004). They compared 36 LP BC_3F_3-derived lines with low saturated fat to the LP parent, CX1834, the NP recurrent parent B019, and a NP cultivar IA3023. They evaluated field emergence, seed germination, phytate P content, and inorganic P content. They found that 15 of the LP lines were not significantly different for field emergence than B019. There was no significant difference for phytate P and inorganic P content of the LP lines compared with CX1834. Their results suggested that breeding of LP soybean cultivars with acceptable field emergence should be possible. However, they indicated that additional research was needed to determine if the improved emergence of the LP lines would be inherited by their progeny when crossed to conventional NP lines.

Chapter 1.

Seed source affects field emergence in low-phytate soybean lines

Materials and Methods

The six BC_3F_4-derived lines with low phytate (LP) used in this study had normal field emergence when evaluated by Spear and Fehr (2007) in Iowa during 2005. They were developed by crossing the LP line CX1834-1-6 (CX1834) to the normal-phytate (NP) line B01769B019 (B019), backcrossing to B019 for three generations, and selecting BC_3F_4 plants from the population that were homozygous for the *pha1* and *pha2* alleles. CX1834 was developed by the USDA-ARS and Purdue University, and B019 was developed jointly by Iowa State University and Pioneer Hi-Bred International, Inc.

Seed of the six LP lines, CX1834, and B019 harvested at Ames in 2005 (IA) by Spear and Fehr (2007) was used to plant two seed increases near Ponce, PR, at the research station of the Illinois Crop Improvement Association where the soil type is a San Antón sandy clay loam (fine-loamy, mixed, superactive, isohyperthermic Cumulic Haplustoll). One seed increase was planted on 16 Oct. 2006 under natural day length conditions and harvested in Jan. 2007 (PR-Jan). The second seed increase was planted on 30 Jan. 2007 under natural day length conditions and harvested in May 2007 (PR-May). For both increases, each line was planted in a single two-row plot 7.6 m long with a seeding rate of 13 seeds m^{-1}.

In the summer of 2007, the eight lines were planted with the IA, PR-Jan, and PR-May sources as a factorial arrangement in a randomized complete-block design with two replications each at Ames, Carlisle, and Lewis, IA. The soil type at Ames is

a Nicollet loam (fine-loamy, mixed, mesic Aquic Hapludoll), at Carlisle is a Tama silty clay loam (fine-silty, mixed, superactive, mesic Typic Agriudoll), and at Lewis is a Marshall silty clay loam (fine-silty, mixed, mesic Typic Hapludoll). The plots were two rows 3.05 m long with spacing of 0.69 m between rows within a plot and 1.02 m between rows of adjacent plots. The seeding rate was 30 seeds m^{-1}. The planting dates were 6 June at Ames, 19 May at Carlisle, and 17 May at Lewis.

Seedling emergence percentage was determined for all plots by counting the number of plants, dividing the number by the 200 seeds planted in each plot, and multiplying the quotient by 100. The plants were counted at the V3 stage, when there were three nodes on the main stem with fully developed leaves (Fehr and Caviness, 1977).

When the field emergence data indicated that there were substantial differences among seed sources, a warm germination test was conducted to make a visual assessment of seed germination from the seed sources. The test was conducted in the Seed Science Center of Iowa State University at Ames with two replications of the eight entries. Two sheets of 19-ply Kimpak® (Neenah, WI) were moistened with 825 mL of water and placed on a 45 x 66 cm fiberglass food service tray. Four random entries were planted with 100 seeds each on a tray. The two trays for each replication were placed in a germination cart 0.5 m wide x 0.7 m deep x 1.6 m high. The cart was made of aluminum, except for the rear panel that was made of Plexiglass™ to permit light from a wall of fluorescent lamps into the cart. The two carts were placed in a growth room at 25°C for 7 d. The classification of seeds for

germination was based on standards provided by the Association of Official Seed Analysts (AOSA, 2005).

The inorganic P and phytic acid P contents of the seed of the eight lines from the three sources were determined. A random sample of 50 seeds of each entry from each source was ground to pass through a 1-mm screen using a UDY Cyclone sample mill (UDY Corporation, Fort Collins, CO). Inorganic P was determined on two replications by a modification of the technique described by Chen et al. (1956). Two replications of 0.5-g of ground seed were independently extracted in 20 mL of 12% trichloroacetic acid that contained 2.6 mM magnesium chloride. The samples were stirred overnight at 4^{o}C. Following extraction, the samples were centrifuged at 14000 x *g* for 20 min. A volume of 100 µL of the solution was added to 3.9 mL of dd$H_2$0 and 4 mL of Chen's reagent was added to the solution. Samples were allowed to react for 2 h at room temperature before they were analyzed at 820 nm on a Varian Cary 50 Bio UV-Visable spectrophotometer (Palo Alto, CA).

Phytic acid P was determined by capillary zone electrophoresis (CZE) as developed by Nardi et al. (1992) and described in detail by Spear and Fehr (2007). A 20-mg sample of ground seed was placed into a scintillation vial and extracted in 10 mL of 0.5 mM L-aspartic acid. The solution was stirred at room temperature for 20 min using a magnetic stir plate. Following extraction, a 750-µL sample was placed in a 0.22 µm Spin-X centrifugal filter (Costar Corning, NY), and centrifuged with a benchtop microfuge for 10 min. A 250 µL aliquot of the filtered sample was loaded on a 96-well plate and analyzed with a Beckman-Coulter P/ACE MDQ capillary electrophoresis system (Fullerton, CA).

The phytate P contents of soybean as measured by CZE are approximately 10-fold less than the values reported for high-performance liquid chromatography (HPLC) analysis (Wilcox et al., 2000; Spear and Fehr 2007). The difference is due at least in part to the acid used to extract phytate P for the two methods. The phytate P for CZE is extracted by L-aspartic acid, which is a weaker acid than the HCl used for extraction of phytate P for HPLC. As a result, less of the phytate P in the seed is solubilized in the CZE procedure. Although the absolute values for phytate P differ for CZE and HPLC, the relative differences between LP and NP lines are similar for the two methods.

Data Analysis

The data for field emergence at the Iowa environments were analyzed as a randomized complete-block design by the linear model procedure of the SAS statistical software (release 9.1.3) (SAS Institute, 2006).

The linear additive model for the analysis of variance across environments for agronomic and seed traits was:

$$Y_{ijkl} = \mu + E_i + RP/E_{(i)j} + S_k + ES_{ik} + G_l + EG_{il} + ESG_{ikj} + \varepsilon_{ijkl}$$

Where;

Y_{ijkl} = the observed value of the l^{th} genotype with the k^{th} source within the j^{th} replication at the i^{th} environment,

μ = the overall mean,

E_i = the effect of the i^{th} environment,

$RP/E_{(i)j}$ = the effect of the j^{th} replication in the i^{th} environment,

S_k = the effect of the k^{th} source,

ES_{jk} = the effect of the interaction between the i^{th} environment and the k^{th} source,

G_j = the effect of the g^{th} genotype,

EG_{il} = the effect of the interaction between the i^{th} environment and the l^{th} genotype,

ESG_{ikj} = the effect of the interaction between the i^{th} environment, the k^{th} source, and the j^{th} genotype, and

ε_{ijkl} = the error of the effect of the $ijkl^{th}$ observation.

Environments and replications within environments were considered random effects and sources and genotypes were considered fixed effects. F-tests were used to determine significance of main effects and interactions. The environment x main effect interactions were used to test the main effects across environments and the environment x source x genotype interaction was used to test the environment x source interactions.

Table 1. Analysis of variance and expected mean squares for field emergence across three Iowa environments in 2007.

Sources of variation	Degrees of freedom	df	Expected mean squares
Environment (E)	e-1	2	$\sigma^2_\varepsilon + eg\sigma^2_r + rg\sigma^2_e$
Replication/E (RP/E)	(r-1)e	3	$\sigma^2_\varepsilon + eg\sigma^2_r$
Source (S)	s-1	2	$\sigma^2_\varepsilon + rg\sigma^2_{es} + reg\phi_s$
E x S	(e-1)(s-1)	4	$\sigma^2_\varepsilon + rg\sigma^2_{es}$

Genotype (G)	g-1	7	$\sigma^2_\varepsilon + rs\sigma^2_{eg} + res\phi_g$
G x E	(g-1)(e-1)	14	$\sigma^2_\varepsilon + rs\sigma^2_{eg}$
S x G	(s-1)(g-1)	14	$\sigma^2_\varepsilon + r\sigma^2_{esg} + re\phi_{sg}$
E x S x G	(e-1)(s-1)(g-1)	28	$\sigma^2_\varepsilon + r\sigma^2_{esg}$
Error	e(r-1)(g-1)(s-1)+e(r-1)(s-1)+(r-1)(g-1)	69	σ^2_ε
Total	ergs-1	143	

The linear additive model for field emergence at each Iowa environment was:

$$Y_{ijk} = u + RP_i + G_j + S_k + GS_{jk} + \varepsilon_{ijk}$$

Where;

Y_{ijk} = the observed value of the j^{th} genotype with the k^{th} source within the i^{th} replication,

μ = the overall mean,

RP_i = the effect of the i^{th} replication,

G_j = the effect of the j^{th} genotype,

S_k = the effect of the k^{th} source, and

ε_{ijk} = the error of the effect of the ijk^{th} observation.

Replications were considered random effects and sources and genotypes were considered fixed effects. F-tests were used to determine significance of main effects and interactions.

Table 2. Analysis of variance and expected mean squares for field emergence at individual Iowa environment in 2007.

Sources of variation	Degrees of freedom	df	Expected mean squares
Replication	r-1	1	$\sigma^2_\varepsilon + g\sigma^2 r$
Genotype	g-1	7	$\sigma^2_\varepsilon + r\Phi_g$
Source	s-1	2	$\sigma^2_\varepsilon + rg\Phi_s$
G x S	(g-1)(s-1)	14	$\sigma^2_\varepsilon + r\Phi_{sg}$
Error	(r-1)(g-1)+(r-1)(s-1)+(r-1)(g-1)(s-1)	23	σ^2_ε
Total	rgs-1	47	

The linear additive model for phytate P and inorganic P was:

$$Y_{ijk} = u + RP_i + G_j + S_k + GS_{jk} + e_{ijk}$$

Where;

Y_{ijk} = the observed value of the j^{th} genotype with the k^{th} source within the i^{th} replication,

μ = the overall mean,

RP_i = the effect of the i^{th} replication,

G_j = the effect of the j^{th} genotype,

S_k = the effect of the k^{th} source, and

ε_{ijk} = the error of the effect of the ijk^{th} observation.

Replications were considered random effects and sources and genotypes were considered fixed effects. F-tests were used to determine significance of main effects and interactions.

Table 3. Analysis of variance and expected mean squares for seed traits

Source of variation	Degrees of freedom	df	Expected mean squares
Replication	r-1	1	$\sigma^2_\varepsilon + g\sigma^2 r$
Genotype	g-1	7	$\sigma^2_\varepsilon + r\Phi_g$
Source	s-1	2	$\sigma^2_\varepsilon + rg\Phi_s$
G x S	(g-1)(s-1)	14	$\sigma^2_\varepsilon + r\sigma^2_{sg}$
Error	(r-1)(g-1)+(r-1)(s-1)+(r-1)(g-1)(s-1)	23	σ^2_ε
Total	rgs-1	47	

Results and Discussion

Significant differences in mean field emergence were observed among the three seed sources (Table 4, Appendix A1). The mean field emergence of all lines was 75.4% for IA, 65.3% for PR-Jan, and 37.8% for PR-May. The differences among sources were due to the major reduction in field emergence of the six A05 lines from the PR-May harvest. In contrast, the emergence of the normal-phytate (NP) parent B01769B019 (B019) was greater than 80% for each of the three sources. The six low-phytate (LP) lines had significantly better emergence than CX1834-1-6 (CX1834) when grown from seed harvested from IA and PR-Jan, but none of the LP

A05 lines were significantly different than CX1834 when grown from the PR-May source. The poor field emergence of the LP lines harvested in PR-May was consistent with the poor field emergence of LP lines and CX1834 harvested from the same location in May 2006 that led to this study.

The number of seeds of the LP lines with normal growth in the warm germination test was noticeably lower for PR-May than for the other two sources (Table 5). In contrast, the NP parent B019 had a higher number of normal seeds for PR-May than the other two sources. Spear and Fehr (unpublished data) found in a warm germination test that CX1834 had 14 percentage units fewer normal seeds compared with B019 for seed from a PR-Jan harvest in 2004 compared with 61 percentage units fewer normal seeds than B019 for seed from a PR-May harvest in 2005. The reduced frequency of seeds of the LP lines with normal growth in this study was associated with the same infection of seed storage fungi reported by Spear and Fehr (2007) for CX1834 in their warm germination test. These results supported their suggestion that susceptibility of LP lines to seed storage fungi may be associated with their reduced field emergence.

The decreased emergence of LP lines from PR-May could not be attributed to differences in the harvest procedures because the seed from all sources was harvested promptly after maturity when the plants were dry enough to thresh. One environmental factor that merits consideration in exploring the cause of the decreased emergence is the temperature during seed fill, which commonly is considered to be the 30 days before physiological maturity. The average daily high temperature during seed fill was 32.4^{o}C for PR-May, 31.1^{o}C for PR-Jan, and 27.9^{o}C for IA. There were

18 d in PR-May, 3 d in PR-Jan, and 1 d in IA when the high daily temperature during seed fill was 32.2°C or greater. The relative humidity for PR-May was less than that of IA. During seed fill, the average high relative humidity was 86.8% in IA, 74.6% in PR-Jan, and 76.3% in PR-May.

The phytate P and inorganic P content of the A05 lines was significantly different for the three sources (Table 4). The mean phytate P content of the six A05 lines was 0.18 mg g^{-1} for IA, 0.41 mg g^{-1} for PR-Jan, and 0.61 mg g^{-1} for PR-May, while their mean inorganic P content was 2.36 mg g^{-1} for IA, 3.97 mg g^{-1} for PR-Jan, and 3.92 mg g^{-1} for PR-May. It is not known if the greater phytate P in seed from PR-May could be associated with the decreased emergence of that source. The results indicated that the environment of seed production can have a major influence on the phytate P and inorganic P content of seed from LP lines. Based on the research of Israel et al. (2007), differences in the P content of the soils of the three seed production environments probably would not be responsible for the significant differences in phytate P among seed sources observed in our study. Additional research will be needed to determine the factors that are responsible for causing such variation among seed sources.

Table 4. Entry means for agronomic and seed traits of six low-phytate lines, the low-phytate donor line, and the normal-phytate recurrent parent from three seed sources.

Entry	Field emergence			Inorganic P			Phytate P		
	IA†	J‡	M§	IA	J	M	IA	J	M
	----------%----------			----------(mg g^{-1})---------			---------(mg g^{-1})----------		
A05-318019	76.0	69.6	26.3	2.15	3.64	3.94	0.16	0.41	0.59
A05-318020	78.0	75.3	25.2	2.02	4.07	3.85	0.30	0.41	0.54
A05-318021	79.9	69.7	18.8	2.48	4.20	4.13	0.21	0.42	0.62
A05-318025	81.0	70.3	31.3	2.57	3.95	3.81	0.15	0.44	0.65
A05-318026	78.8	68.2	23.2	2.34	3.87	4.50	0.15	0.46	0.63
A05-318031	71.9	67.3	27.3	2.63	4.10	3.29	0.14	0.32	0.62
CX1834-1-6	54.5	38.8	20.3	2.21	4.01	4.38	0.41	0.32	0.61
B01769B019	81.7	80.3	82.0	0.27	0.30	0.23	0.92	1.86	1.68
$LSD_{0.05}$	10.8	8.0	12.5	0.17	0.22	0.46	0.17	0.16	0.22

† Iowa 2005 harvest source

‡ Puerto Rico January 2007 harvest source

§ Puerto Rico May 2007 harvest source

Table 5. Mean germination percentages from a warm germination test of six low-phytate lines, the low-phytate donor line, and the normal-phytate recurrent parent from three sources.

Entry	Iowa 2005 harvest				Puerto Rico January 2007 harvest				Puerto Rico May 2007 harvest			
	Normal†	Abnorm‡	Dead§	HardSd¶	Normal	Abnorm	Dead	HardSd	Normal	Abnorm	Dead	HardSd
	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------
						%						
A05-318019	40.0	49.0	10.0	0.5	45.5	42.5	11.0	1.0	16.0	48.5	35.5	0.0
A05-318020	54.0	36.5	9.5	0.0	57.5	32.5	9.0	1.0	20.5	67.5	11.5	0.5
A05-318021	34.5	57.5	7.0	1.0	45.5	49.5	4.5	0.5	19.5	47.5	33.0	0.0
A05-318025	44.5	43.5	12.0	0.0	52.0	42.0	6.0	0.0	19.5	67.5	12.0	1.0
A05-318026	34.0	51.5	14.5	0.0	60.0	37.0	3.0	0.0	15.0	61.5	23.0	0.5
A05-318031	34.0	61.5	4.5	0.0	62.0	33.0	5.0	0.0	25.5	55.5	17.5	1.5
CX1834-1-6	43.5	39.0	17.5	0.0	35.0	45.0	20.0	0.0	16.0	39.5	44.5	0.0
B01769B019	58.5	31.5	10.0	0.0	73.5	26.0	0.5	0.0	84.0	13.5	2.0	0.5
$LSD_{0.05}$	15.9	22.1	15.8	1.2	19.7	20.6	7.4	1.9	15.4	20.9	30.6	1.2

† Seeds that emerged and continued to show growth.

‡ Seeds that had the radical emerge, but growth ceased.

§ Seeds that never grew radicals.

¶ Seeds that never imbibed water.

The results have important implications for the choice of environments that are most suitable for breeding LP cultivars with the *pha*1 and *pha*2 alleles and for commercial production of their seed. It is common in soybean breeding programs to grow segregating populations and seed increases in subtropical environments during the winter in North America. If segregating populations are grown in environments comparable to PR-May, there could be a substantial decrease in the frequency of the LP segregates that would be recovered in subsequent generations. The PR-May type environments also would not be useful for obtaining seed of experimental LP lines for evaluation of field emergence, when the goal is to develop LP lines that would have normal field emergence when grown from a source comparable to IA or PR-Jan.

The field emergence of the A05 lines when grown from the IA source was similar to that reported by Spear and Fehr (2007). This study supported their results that indicated it is possible to develop LP lines with field emergence similar to NP lines, but only when they are grown from appropriate seed sources. The two studies indicate that it may be possible to grow LP cultivars with adequate field emergence as long as the seed for commercial plantings is produced in temperate climates and seed lots are adequately tested for germination before planting. Additional research will be needed to determine the range of environments in which seed of LP cultivars can be produced successfully and the types of germination tests that should be used to identify acceptable seed lots (Meis et al., 2003; Spear and Fehr, 2007).

CHAPTER 2.

FIELD EMERGENCE FOR LOW-PHYTATE SOYBEAN LINES FROM SINGLE-CROSS AND BACKCROSS POPULATIONS

Materials and Methods

Development of populations segregating for low phytate and 1% linolenic acid

The low-phytate (LP) lines chosen for the study were A05-218007, A05-314030, and A05-318025. A05-218007 was a LP line with 1% linolenic acid in its seed oil that had a mean of 83% field emergence in 10 replications grown at the Iowa State University Agronomy Research Farm in 2005. The 1% linolenic acid trait was controlled by the alleles *fan1(A5), fan2*, and *fan3* (Fehr et al., 1992). A05-218007 was a BC_3F_3-derived line selected from a backcross population in which CX1834-1-6 (CX1834) was the donor of the LP trait and IA2064, a normal-phytate (NP) cultivar with 1% linolenic acid, was the recurrent parent. A05-314030 was a LP line with 1% linolenic acid that had a mean of 74% field emergence over five locations grown in Iowa during 2005. A05-314030 was a BC_2F_3-derived line selected from the backcross population in which CX1834 was the donor of the LP trait and IA3017, a NP cultivar with 1% linolenic acid, was the recurrent parent. A05-318025 was a LP BC_3F_4-derived line low in saturated fatty acids selected from the backcross population in which CX1834 was the donor of the LP trait and B01769B019 (B019), a NP line with low saturated fatty acids, was the recurrent parent.

A05-318025 was crossed to A04-542015 to form the population designated AX20196 (Pop 1UL). A05-218007 was crossed to A04-642005 to form the population AX20197 (Pop 2UL). A05-314030 was crossed to A04-442033 to form

the population AX20200 (Pop 3UL). A04-442033, A04-542015, and A04-642005 were NP lines with 1% linolenic acid developed at Iowa State University. The crosses were made at the Agricultural Engineering and Agronomy Research Center near Ames, IA, in July 2005. The soil type at Ames is a Nicolett loam (fine-loamy, mixed mesic Aquic Hapludoll).

The F_1 seeds and seeds of the NP parents A04-442033, A04-542015 and A04-642005 were planted at the Illinois Crop Improvement Association (ICIA) research station near Ponce, PR, in October 2005. The soil type at Ponce is a San Antón sandy clay loam (fine-loamy, mixed, superactive, isohyperthermic Cumulic Haplustoll). To obtain suitable flowers for crossing, the F_1 plants were grown under artificial lighting to extend day length. The F_1 plants were backcrossed to their NP parent in November 2005. The BC_1F_1 seeds were harvested separately for each cross, and the F_1 plants were harvested individually to obtain F_2 seed. The BC_1 populations will be designated hereinafter as Pop 1UL-BC, Pop 2UL-BC, and Pop 3UL-BC.

Development of populations segregating for low phytate and low saturated fatty acids

The LP lines with low saturated fatty acid content chosen for this study were A05-318011, A05-318020, and A05-318025, which were BC_3F_4-derived lines selected from backcrossing the LP trait from CX1834 into B019. A05-318011 had a mean field emergence of 70.6%, A05-318020 had 75.2%, and A05-318025 had 74.6% averaged over five Iowa locations in 2005 (Spear and Fehr, 2007). A05-318011 was crossed to the cultivar IA2070 to form the population AX20191 (Pop

4LS), A05-318020 was crossed to the cultivar IA2069 to form AX20192 (Pop 5LS), and A05-318025 was crossed to IA2069 to form AX20193 (Pop 6LS). IA2069 and IA2070 were low-saturate cultivars developed at Iowa State University. The crosses were made at the Agricultural Engineering and Agronomy Research Center near Ames, IA, in July 2005.

The F_1 seeds and seeds of the recurrent parents IA2069 and IA2070 were planted at the ICIA research station near Ponce, PR, in October, 2005. The F_1 plants were backcrossed to the NP parent in November 2005. The BC_1F_1 seeds were harvested separately for each cross. The BC_1 populations will hereinafter be designated Pop 4LS-BC, Pop 5LS-BC, and Pop 6LS-BC. The F_1 plants were harvested individually to obtain F_2 seed.

Development of LP lines with 1% linolenic acidor low saturated fat

Individual F_2 seeds obtained from F_1 plants of Pop 1UL, Pop 2UL Pop 3UL, Pop 4LS, Pop 5LS, and Pop6 LS grown at Ponce, PR, were tested for phytate content at Iowa State University in January 2006 by a technique adapted from Wilcox et al. (2000). To obtain 40 LP F_2 seeds with the *pha1pha1pha2pha2* genotype for each population, it was necessary to evaluate 820 seeds of each population based on the formula provided by Sedcole (1977). Each seed was split into two parts with a razor blade. The one-third of each seed without the embryonic axis was used for testing and the remaining two-thirds of the seed were saved for planting. The one-third part of the seed was placed in an individual packet and crushed with a steel weight. The crushed sample was placed in a 12 x 75 mm glass tube. An aliquot of 1 mL of 12.5%

(w/v) TCA and 25 m*M* $MgCl_2$ was added to the test tube to extract the inorganic P. After 10 min, 1 mL of Chen's Reagent was added to the mixture. Chen's Reagent consisted of 1 volume of 3 *M* H_2SO_4, 1 volume 10% (w/v) ascorbic acid, 1 volume 20 m*M* ammonium molybdate, and 2 volumes DD H_2O. The samples were allowed to sit at room temperature for 10 min. Samples were scored as LP if the solution turned dark blue and NP if the solution remained clear to light blue.

The BC_1F_1 produced in November 2005 and the selected LP F_2 seeds were planted at Ponce, PR, in January 2006. The plants were grown under artificial lighting to maximize seed production. The F_2 and BC_1F_1 plants were harvested individually from each population during May. Pop 2UL had 16 F_2 plants, Pop 3UL had 22, Pop 4LS had 20, and Pop 5LS had 13. Pop 1UL-BC had 43 BC_1F_1 plants, Pop 2UL-BC had 39, Pop 3UL-BC had 9, Pop 4LS-BC had 29, Pop 5LS-BC had 35, and Pop 6LS-BC had 32. Five individual F_3 seeds were tested from each F_2 plant to be 95% certain that the plant was homozygous for the *pha* alleles (Sedcole, 1977). Pop 2UL had 7 confirmed LP plants, Pop 3UL had 4, Pop 4LS had 14, and Pop 5LS had 7. No LP Pop 1UL or Pop 6LS plants were recovered.

BC_1F_2 seeds were split and tested for phytate content according the procedure described above. Pop 1UL-BC had 6 LP BC_1F_2 seeds and Pop 2UL-BC had 6. Pop 3UL-BC had no LP seeds and was dropped from further study. Pop 5LS-BC had 13 LP seeds and Pop 6LS-BC had 18. Pop 4LS had no LP seeds and was dropped from further study.

The LP $F_{2:3}$ lines were grown at two Iowa locations in 2006 with two replications of a randomized complete-block design at each location. There were 50

entries in the test (Table 3). The locations were the Agronomy Farm and the Burkey Farm near Ames, IA. The soil type at both locations is a Nicolett loam (fine-loamy, mixed mesic Aquic Hapludoll). The plots were one-row 0.76 m long with spacing of 1.02 m between rows and 1.07 m between the ends of rows. Each plot was planted with 20 seeds, or as many seeds as were available. The planting dates were 17 May at the Agronomy Farm and 18 May at the Burkey Farm.

The plots of the $F_{2:3}$ lines and checks were evaluated for seedling emergence at the V3 stage, when the second trifoliate leaf above the unifoliate node is fully developed (Fehr and Caviness, 1977). After the emergence data were collected, the plots were thinned to a maximum of eight plants to promote increased seed production on individual plants. Maturity was recorded for both $F_{2:3}$ and BC_1F_2 plots as days after 31 August when 95% of the pods in a plot have reached their mature color. The plants from each plot were classified for maturity and harvested and threshed individually.

Five individual F_4 seeds were tested from one random F_3 plant of each $F_{2:3}$ line to be 95% certain that the plant was homozygous for the *pha* alleles (Sedcole, 1977). A five-seed bulk from the same random F_3 plant was evaluated for fatty ester content by gas chromatography using the method described by Hammond (1991). If the first F_3 plant was not homozygous for LP or did not have the desired fatty acid composition additional plants from a plot were evaluated. The desired linolenic oil content for UL populations was less than 1.6% linolenic acid and the desired saturated oil content for LS populations was less than 8.5% saturated oils. If plants

within each of the lines were not identified that contained both the LP phenotype and the desired fatty acid composition, they were dropped from the study.

The LP BC_1F_2 seeds were grown at the Agronomy Farm in 2006. In addition, there were BC_1F_2 seeds that were not tested due to time constraints during the spring planting season. These BC_1F_2 seeds were planted in progeny rows to obtain single plants that could be evaluated for their phytate content. The plants were classified for maturity and harvested individually. Five individual BC_1F_3 seeds from each BC_1F_2 plant were tested from to be 95% certain that the plant was homozygous for the *pha* alleles (Sedcole 1977).

Seed from the one F_3 plant from each LP $F_{2:3}$ line and each LP BC_1F_2 plant were planted in progeny rows for seed increase at Ponce, PR, during October 2006, as well as the parental lines. The plots were harvested in bulk. Due to adverse environmental conditions, inadequate numbers of seed were obtained. Seed of the $F_{3:5}$ lines, $BC_1F_{2:4}$ lines, LP parent lines, and NP parent lines was replanted in Puerto Rico during January 2007. Acceptable seed quantities were obtained from the Puerto Rico May 2007 harvest.

Sixteen $F_{3:6}$ and 33 $BC_1F_{2:5}$ lines, six NP parent lines, and four LP parent lines were planted at three Iowa locations in 2007 with two replications of a randomized complete-block design at each location. The locations were Ames, Carlisle, and Lewis. The plots were two-rows 3.05 m long with spacing of 0.69 m between rows within a plot and 1.02 m between rows of adjacent plots. The seeding rate was 200 seeds per plot (30 seeds m^{-1}). Data were collected on all plots at all locations for

seedling emergence. Seedling emergence was determined by counting the number of plants in each plot at the V3 stage.

Data Analysis

The data for field emergence at the Iowa environments were analyzed as a randomized complete-block design by the linear model procedure of the SAS statistical software (release 9.1.3) (SAS Institute, 2006).

The linear additive model for the analysis of variance across environments for emergence on an entry basis was:

$$Y_{ijk} = \mu + E_i + RP/E_{(i)j} + G_l + EG_{ik} + \varepsilon_{ijk}$$

Where;

Y_{ijk} = the observed value of the k^{th} genotype within the j^{th} replication at the i^{th} environment,

μ = the overall mean,

E_i = the effect of the i^{th} environment,

$RP/E_{(i)j}$ = the effect of the j^{th} replication in the i^{th} environment,

G_k = the effect of the g^{th} genotype,

EG_{ik} = the effect of the interaction between the i^{th} environment and the l^{th} genotype, and

ε_{ijk} = the error of the effect of the ijk^{th} observation.

Environments and replications within environments were considered random effects and genotypes were considered fixed effects. F-tests were used to determine

the significance of main effects and interactions. The environment x main effect interactions were used to test the main effects across environments.

Table 2. Analysis of variance and expected mean squares on an entry basis across three Iowa environments in 2007.

Sources of variation	Degrees of freedom	df	Expected mean squares
Environment (E)	e-1	2	$\sigma^2_\varepsilon + g\sigma^2_r + rg\sigma^2_e$
Replication/E (RP/E)	(r-1)e	3	$\sigma^2_\varepsilon + g\sigma^2_r$
Genotype (G)	g-1	59	$\sigma^2_\varepsilon + r\sigma^2_{eg} + re\Phi_g$
Single-cross LP lines	sc-1	15	$\sigma^2_\varepsilon + r\sigma^2_{eg} + re\Phi_{sc}$
Backcross LP lines	bc-1	32	$\sigma^2_\varepsilon + r\sigma^2_{eg} + re\Phi_{bc}$
NP parents	np-1	4	$\sigma^2_\varepsilon + r\sigma^2_{eg} + re\Phi_{np}$
LP parents	lp-1	5	$\sigma^2_\varepsilon + r\sigma^2_{eg} + re\Phi_{lp}$
SC vs. BC		1	
SC + BC vs. NP parents		1	
NP vs. LP parents		1	
G x E	(g-1)(e-1)	108	$\sigma^2_\varepsilon + r\sigma^2_{eg}$
Error	e(r-1)(g-1)	177	σ^2_ε
Total	erg-1	359	

The linear additive model for field emergence at each Iowa environment was:

$$Y_{ij} = u + RP_i + G_j + \varepsilon_{ij}$$

Where;

Y_{ij} = the observed value of the j^{th} genotype within the i^{th} replication,

μ = the overall mean,

RP_i = the effect of the i^{th} replication,

G_j = the effect of the j^{th} genotype, and

ε_{ij} = the error of the effect of the ij^{th} observation.

Table 3. Analysis of variance for field emergence at each Iowa environment in 2007.

Sources of variation	Degrees of freedom	df	Expected mean squares
Replications	r-1	1	$\sigma^2_\varepsilon + g\sigma^2_r$
Genotype	g-1	59	$\sigma^2_\varepsilon + r\Phi_g$
Single-cross LP lines	sc-1	15	$\sigma^2_\varepsilon + r\Phi_{sc}$
Backcross LP lines	bc-1	32	$\sigma^2_\varepsilon + r\Phi_{bc}$
NP parents	np-1	5	$\sigma^2_\varepsilon + r\Phi_{np}$
LP parents	lp-1	4	$\sigma^2_\varepsilon + r\Phi_{lp}$
SC vs. BC		1	
SC + BC vs. NP parents		1	
NP vs. LP parents		1	
Error	(r-1)(g-1)	59	σ^2_ε
Total	rg-1	119	

Results and Discussion

There were significant differences in field emergence among the 60 entries at the three test environments and combined across environments (Tables 4 and 5). The difference in the mean field emergence at the three environments of 28.7% at Ames, 41.6% at Lewis, and 48.5% at Carlisle was significant at the 5% probability level. The genotype x environment interaction was significant due to a change in the rank of entries and the relative differences among entries at the three environments. For example, IA2069 has greater emergence than IA2070 at Ames and Carlisle, but lower emergence at Lewis.

There were significant differences for field emergence within and among the single-cross (SC) lines, backcross (BC) lines, LP parents, and NP parents (Tables 4, 5, and 6). The SC lines had a mean field emergence of 42.8% and a range of 6.9 to 69.8%, the BC lines had a mean of 31.4% and a range of 5.5 to 55.5%, the four LP parents had a mean of 42.0% and a range of 30.1 to 59.0%, and five of the six NP parents had a mean of 76.5% and a range of 71.9 to 80.8%. A04-642005 was not included in the mean of the NP parents and was not used for comparison with the LP, SC, and BC lines because of its poor emergence, for which there was no explanation. There was a difference of 25.5 percentage units in field emergence between the mean of the LP, SC, and BC lines (51.0%) and the mean of five of the NP parents (76.5%) (Table 6). Only three of the 49 LP, SC, and BC lines were not significantly lower in emergence than the mean of five NP parents. The emergence of the SC lines 519007 (69.3%) and 519002 (67.1%) from Pop 4LS and the SC line 519014 (69.8%) from Pop 2UL was not significantly different than IA2070 (75.2%), A04-442033 (71.3%),

and A05-542015 (74.3%) based on the $LSD_{0.01}$. This suggests that it may be possible to select for lines with improved field emergence when grown from seed produced in environments similar to the PR-May type.

This study was conducted to determine if the improved emergence of LP lines identified by Spear and Fehr (2007) would be inherited by their progeny. The research was initiated before the major influence of seed source on the field emergence of LP lines was known. Two of the lines used in this study that Spear and Fehr (2007) identified that were A05-318020 and A05-318025. When evaluated with the IA seed source, the field emergence of A05-318020 (78.0%) and A05-318025 (81.0%) was significantly greater than the donor parent CX1834 (54.5%) and not significantly less than B019 (81.7%), which was consistent with the results of Spear and Fehr (2007) (Chapter 1 – Table 4). However, when the two lines were evaluated with the PR-May source, their emergence was not superior to CX1834 (Table 6). These results indicated that any assessment of genetic improvement for field emergence of LP lines is dependent on the source of seed used to evaluate the parent lines and their progeny. Based on the use of PR-May seed, no genetic improvement had been made in the field emergence of the LP lines studied by Spear and Fehr (2007), which made it impossible to evaluate if improved emergence of LP lines would be inherited by their progeny. Although the original objective of the research was not possible to evaluate, it was noteworthy that some of the 49 SC and BC lines evaluated had improved emergence compared with CX1834. It will be necessary to test the three lines again with seed from another PR-May harvest to determine if their performance is repeatable when grown from that type of a seed source. If the lines

have improved emergence when grown from that source, it may be possible with sufficient breeding to overcome the impact of the PR-May type of environment on the field emergence of LP lines.

Additional research will be needed to determine if the improved emergence of LP lines grown from seed produced in Iowa, such as A05-318020 and A05-318025, will be inherited by their progeny. The seed harvested from this experiment at Ames, Carlisle, and Lewis could be used to conduct the research. If their improved emergence is inherited by their progeny, breeding cultivars with normal field emergence should be possible. The seed for their commercial production would have to be grown in environments similar those of Iowa.

Both low-saturate and low-linolenic oil lines with field emergence comparable to the NP checks were identified. These results agree with Spear and Fehr (2007) that it is possible to select for lines that have both the LP and a modified oil phenotype with improved field emergence.

The difference in the mean emergence of the SC and BC lines of 11.4 percentage units was highly significant ($P<0.01$). BC lines performed worse at every location. At Ames, BC lines had 10.1 percentage units less, at Carlisle 9.9, and at Lewis 14.3. There was no obvious explanation for this difference between the two types of lines. The additional backcrossing to a NP parent would have been expected to improve field emergence of the BC lines compared with that of the SC lines.

Table 4. Analysis of variance for field emergence percentage of 60 lines across three Iowa environment in 2007.

Sources of variation	df	Mean squares
Environment	2	12170.1*
Replication/E	3	1276.7
Genotype	59	2136.6**
Single-cross LP lines	15	2164.1**
Backcross LP lines	32	1058.9**
LP parents	4	816.4**
NP parents	5	995.2**
SC vs. BC	1	8427.6**
SC + BC vs. NP parents	1	13287.3**
LP vs. NP parents	1	1500.2**
Genotype x environment	118	98.9**
Error	177	48.3
CV (%)		17.6

* significant at the 0.05 probability level
** significant at the 0.01 probability level

Table 5. Analyses of variances for field emergence of 60 lines at each Iowa environment in 2007.

		Mean squares		
Sources of variation	df	Ames	Carlisle	Lewis
Replication	1	1840.8**	1098.1**	891.1**
Genotype	59	523.2**	881.7**	929.7**
Single-cross LP lines	15	495.1**	889.0**	957.4**
Backcross LP lines	32	258.9**	525.9**	465.7**
LP parents	4	309.7**	509.1*	218.5*
NP parents	5	776.0**	448.3**	348.6**
SC vs. BC	1	2181.3**	2091.1**	4431.9**
LP vs. NP parents	1	182.9*	407.3**	1114.1**
SC + BC vs. NP parents	1	2549.4**	5149.2**	5991.5**
Error	59	36.4	50.5	58.1
CV (%)		21.0	14.6	18.3

* significant at the 0.05 probability level
** significant at the 0.01 probability level

Table 6. Field emergence percentage for 16 single-cross LP, 33 backcross LP, four LP parent lines, and six NP parent lines evaluated at three Iowa environments in 2007.

Entry	Ames			Carlisle			Lewis			Overall mean	Line designation	
	Rep 1	Rep 2	Mean	Rep 3	Rep 4	Mean	Rep 5	Rep 6	Mean		Line type	Population
	----------									%----------		
519001	52.5	25.5	39.0	56.0	49.0	52.5	49.0	61.5	55.3	48.9	SC†	Pop 4LS§
519002	59.5	40.5	50.0	80.0	74.5	77.3	71.0	77.0	74.0	67.1	SC	Pop 4LS
519003	26.5	25.5	26.0	54.5	38.5	46.5	39.5	45.0	42.3	38.3	SC	Pop 4LS
519004	9.0	2.5	5.8	17.5	11.5	14.5	15.5	15.5	15.5	11.9	SC	Pop 4LS
519005	26.5	18.0	22.3	22.5	22.5	22.5	21.0	28.5	24.8	23.2	SC	Pop 4LS
519006	25.0	15.0	20.0	43.5	59.0	51.3	28.0	28.0	28.0	33.1	SC	Pop 4LS
519007	64.5	46.5	55.5	69.0	83.0	76.0	71.5	81.0	76.3	69.3	SC	Pop 4LS
519008	40.5	32.0	36.3	38.5	43.5	41.0	27.5	49.0	38.3	38.5	SC	Pop 4LS
519009	50.5	32.5	41.5	53.0	54.0	53.5	41.5	53.0	47.3	47.4	SC	Pop 4LS
519010	5.5	3.5	4.5	6.5	10.5	8.5	6.5	9.0	7.8	6.9	SC	Pop 4LS
519011	41.5	28.5	35.0	68.0	60.0	64.0	46.5	60.5	53.5	50.8	SC	Pop 4LS
519012	17.0	15.5	16.3	45.0	35.5	40.3	19.0	33.5	26.3	27.6	SC	Pop 5LS¶
519013	57.5	37.5	47.5	65.5	52.5	59.0	48.5	56.5	52.5	53.0	SC	Pop 5LS
519014	58.5	43.5	51.0	82.0	68.5	75.3	82.0	84.0	83.0	69.8	SC	Pop 2UL#
519015	25.5	22.0	23.8	56.5	63.0	59.8	51.5	45.0	48.3	43.9	SC	Pop 2UL
519016	35.5	40.0	37.8	76.0	58.5	67.3	51.0	75.0	63.0	56.0	SC	Pop 2UL
519017	50.0	27.5	38.8	81.0	69.0	75.0	56.5	70.0	63.3	59.0	LP parent	A04-314030
519018	37.5	28.0	32.8	58.5	47.5	53.0	47.5	57.5	52.5	46.1	NP	A04-642005
519019	84.5	64.5	74.5	95.0	86.5	90.8	76.5	78.0	77.3	80.8	NP	IA2069
519020	68.0	46.5	57.3	90.5	65.5	78.0	90.0	90.5	90.3	75.2	NP	IA2070
519021	31.5	16.5	24.0	29.0	33.0	31.0	32.5	38.0	35.3	30.1	LP parent	A05-318020
519022	58.5	48.0	53.3	86.5	88.0	87.3	68.5	82.0	75.3	71.9	NP	A04-442033
519023	17.0	9.0	13.0	65.0	38.5	51.8	44.0	48.0	46.0	36.9	LP parent	A05-318025
519024	65.0	45.0	55.0	92.0	81.5	86.8	75.0	87.0	81.0	74.3	NP	A05-542015

Table 6. Cont.

Entry	Ames			Carlisle			Lewis			Overall mean	Line designation	
	Rep 1	Rep 2	Mean	Rep 3	Rep 4	Mean	Rep 5	Rep 6	Mean		Line type	Population
	----------					----%----------						
519025	52.0	37.5	44.8	60.5	60.0	60.3	52.0	54.5	53.3	52.8	LP parent	CX1834-1-6
519026	35.0	26.5	30.8	60.5	43.5	52.0	58.0	50.5	54.3	45.7	LP parent	CX1834-1-6
519027	41.0	35.5	38.3	47.5	33.0	40.3	33.0	31.5	32.3	36.9	BC_1‡	Pop 1UL-BC††
519028	29.5	32.5	31.0	63.0	51.5	57.3	33.5	39.5	36.5	41.6	BC_1	Pop 1UL-BC
519029	15.0	11.5	13.3	38.5	35.5	37.0	31.0	20.5	25.8	25.3	BC_1	Pop 2UL-BC‡‡
519030	19.0	14.0	16.5	64.0	34.0	49.0	39.5	47.0	43.3	36.3	BC_1	Pop 2UL-BC
519031	17.0	12.5	14.8	34.5	41.5	38.0	24.0	35.5	29.8	27.5	BC_1	Pop 2UL-BC
519032	25.5	13.0	19.3	50.0	47.5	48.8	45.0	52.5	48.8	38.9	BC_1	Pop 2UL-BC
519033	16.0	7.5	11.8	55.5	55.0	55.3	33.5	24.0	28.8	31.9	BC_1	Pop 2UL-BC
519034	50.0	23.0	36.5	63.5	55.0	59.3	48.5	47.0	47.8	47.8	BC_1	Pop 2UL-BC
519035	42.0	37.0	39.5	72.5	45.5	59.0	49.5	55.5	52.5	50.3	BC_1	Pop 5LS-BC§§
519036	49.0	42.5	45.8	65.5	68.0	66.8	50.5	57.5	54.0	55.5	BC_1	Pop 5LS-BC
519037	38.0	27.0	32.5	50.0	46.0	48.0	32.5	37.0	34.8	38.4	BC_1	Pop 5LS-BC
519038	16.5	14.0	15.3	29.5	28.0	28.8	24.5	25.0	24.8	22.9	BC_1	Pop 5LS-BC
519039	24.5	18.0	21.3	31.5	34.0	32.8	25.5	26.0	25.8	26.6	BC_1	Pop 5LS-BC
519040	16.5	15.5	16.0	49.0	22.5	35.8	14.5	22.0	18.3	23.3	BC_1	Pop 5LS-BC
519041	16.0	18.5	17.3	31.5	30.5	31.0	23.0	16.5	19.8	22.7	BC_1	Pop 5LS-BC
519042	18.5	32.5	25.5	54.0	41.0	47.5	27.5	47.0	37.3	36.8	BC_1	Pop 5LS-BC
519043	21.0	19.5	20.3	49.5	39.5	44.5	36.5	99.0	67.8	44.2	BC_1	Pop 5LS-BC
519044	41.5	27.0	34.3	64.5	71.0	67.8	44.5	60.5	52.5	51.5	BC_1	Pop 5LS-BC
519045	4.0	5.0	4.5	4.5	4.5	4.5	9.5	5.5	7.5	5.5	BC_1	Pop 5LS-BC
519046	14.5	14.5	14.5	20.5	10.0	15.3	10.5	8.0	9.3	13.0	BC_1	Pop 6LS-BC¶¶
519047	13.0	17.0	15.0	18.5	19.5	19.0	18.5	21.0	19.8	17.9	BC_1	Pop 6LS-BC
519048	37.5	24.0	30.8	46.5	43.5	45.0	31.5	44.0	37.8	37.8	BC_1	Pop 6LS-BC

Table 6. Cont.

	Ames			Carlisle			Lewis				Line designation	
Entry	Rep 1	Rep 2	Mean	Rep 3	Rep 4	Mean	Rep 5	Rep 6	Mean	Overall mean	Line type	Population
	----------					%----------						
519049	11.5	8.5	10.0	23.0	16.0	19.5	22.5	15.5	19.0	16.2	BC_1	Pop 6LS-BC
519050	15.5	17.5	16.5	29.5	24.5	27.0	17.5	22.0	19.8	21.1	BC_1	Pop 6LS-BC
519051	16.5	14.0	15.3	30.5	32.5	31.5	18.5	23.0	20.8	22.5	BC_1	Pop 6LS-BC
519052	9.0	15.5	12.3	34.0	24.0	29.0	13.0	12.5	12.8	18.0	BC_1	Pop 6LS-BC
519053	23.5	20.0	21.8	59.0	47.0	53.0	30.0	23.0	26.5	33.8	BC_1	Pop 6LS-BC
519054	8.0	4.0	6.0	10.0	23.5	16.8	12.0	15.5	13.8	12.2	BC_1	Pop 6LS-BC
519055	8.0	6.5	7.3	27.5	19.5	23.5	10.5	9.0	9.8	13.5	BC_1	Pop 6LS-BC
519056	22.0	17.0	19.5	46.5	33.0	39.8	28.0	29.0	28.5	29.3	BC_1	Pop 6LS-BC
519057	22.0	18.0	20.0	61.0	44.0	52.5	42.5	40.0	41.3	37.9	BC_1	Pop 6LS-BC
519058	36.5	35.0	35.8	60.5	57.0	58.8	39.5	55.0	47.3	47.3	BC_1	Pop 6LS-BC
519059	68.5	53.5	61.0	96.0	91.5	93.8	86.5	85.0	85.8	80.2	NP	B01769B019
519060	55.0	37.5	46.3	59.5	64.5	62.0	53.5	48.0	50.8	53.0	BC_1	Pop 5LS-BC
Mean			28.7			48.5			41.6	39.6		
SE			6.0			7.1			7.6	7.0		
$LSD_{0.05}$			12.1			14.2			15.2	7.9		
$LSD_{0.01}$			16.0			18.9			20.3	10.4		

† Single-cross lines

‡ Backcross lines

§ A05-318011 x IA2070

¶ A05-318020 x IA2069

A05-218007 x A04-642005

†† (A05-318025 x A04-542015) x A04-542015

‡‡ (A05-218007 x A04-642005) x A04-642005

§§ (A05-318020 x IA2069) x IA2069

¶¶ (A05-318025 x IA2069) x IA2069

APPENDIX A

ANALYSES OF VARIANCE FOR FIELD EMERGENCE, WARM GERMINATION, AND SEED COMPOSITION TRAITS FOR CHAPTER 1.

Table A1. Analysis of variance for field emergence percentage of eight lines planted with three seed sources in three Iowa environments in 2007.

Sources of variation	df	Mean squares
Environment	2	1811.9**
Replication/E	3	223.7
Genotype	7	2534.9*
Genotype x environment	14	56.1ns
Source	2	25298.3**
Source x environment	4	144.4ns
Source x genotype	14	730.2**
Source x environment x genotype	28	58.4ns
Error	110	78.5
CV (%)		15.3

* significant at the 0.05 probability level

** significant at the 0.01 probability level

ns = not significant at the 0.05 probability level

Table A2. Analysis of variances for field emergence of eight lines planted with three seed sources at each Iowa environment in 2007.

		Mean squares		
Sources of variation	df	Ames	Carlisle	Lewis
Replication	1	51.1ns	182.1ns	438.0**
Genotype	7	762.7**	1151.7**	732.7**
Source	2	9830.6**	6326.6**	9429.9**
Source x genotype	14	335.9*	288.4**	222.7**
Error	23	122.1	82.1	31.2
CV (%)		19.9	17.0	8.6

* significant at the 0.05 probability level

** significant at the 0.01 probability level

ns = not significant at the 0.05 probability level

Table A3. Analyses of variance for seed traits of eight lines from three seed sources.

		Mean squares		
Sources of variation	df	Warm germ	Phytic acid P	Inorganic P
Replication	1	784.1**	0.1**	1.1**
Genotype	7	1028.5**	0.9**	7.6**
Source	2	2920.8**	0.8**	10.9**
Source x genotype	14	257.3**	0.1**	0.4**
Error	23	49.6	0.004	0.02
CV (%)		17.1	12.7	4.6

** significant at the 0.01 probability level

APPENDIX B

MEAN PERFORMANCE OF EACH ENTRY AT EACH LOCATION FOR CHAPTER 1.

Table B1. Field emergence percentage of six low-phytate lines, the low-phytate donor, and the normal-phytate recurrent parent planted with three seed sources at three Iowa environments in 2007.

	Ames			Carlisle			Lewis				
Entry	Rep 1	Rep 2	Mean	Rep 3	Rep 4	Mean	Rep 5	Rep 6	Mean	Overall mean	Source
	----------	----------	----------	----------	----------	%----------	----------	----------	----------	----------	
A05-318019	76.5	73.5	75.0	69.5	74.0	71.8	77.0	85.5	81.3	76.0	IA†
A05-318020	81.0	77.0	79.0	61.5	75.0	68.3	84.0	89.5	86.8	78.0	IA
A05-318021	79.5	69.0	74.3	79.0	77.0	78.0	83.5	91.5	87.5	79.9	IA
A05-318025	77.0	93.0	85.0	85.5	59.0	72.3	82.0	89.5	85.8	81.0	IA
A05-318026	80.5	69.0	74.8	79.5	64.0	71.8	88.5	91.5	90.0	78.8	IA
A05-318031	78.0	78.5	78.3	76.5	32.5	54.5	74.5	91.5	83.0	71.9	IA
CX1834-1-6	61.5	47.0	54.3	44.5	37.5	41.0	69.0	67.5	68.3	54.5	IA
B01769B019	80.0	70.0	75.0	76.0	81.5	78.8	90.0	92.5	91.3	81.7	IA
A05-318019	77.5	71.0	74.3	67.0	57.0	62.0	67.5	77.5	72.5	69.6	PR-Jan‡
A05-318020	77.0	69.0	73.0	72.5	82.5	77.5	68.5	82.0	75.3	75.3	PR-Jan
A05-318021	83.0	73.0	78.0	57.0	58.0	57.5	70.0	77.0	73.5	69.7	PR-Jan
A05-318025	76.0	64.0	70.0	59.0	64.5	61.8	82.0	76.5	79.3	70.3	PR-Jan
A05-318026	67.5	77.5	72.5	65.5	57.0	61.3	66.0	75.5	70.8	68.2	PR-Jan
A05-318031	62.0	68.0	65.0	67.5	63.5	65.5	64.0	79.0	71.5	67.3	PR-Jan
CX1834-1-6	28.0	40.5	34.3	27.0	32.0	29.5	46.0	59.5	52.8	38.8	PR-Jan
B01769B019	69.5	80.0	74.8	81.0	82.0	81.5	81.5	88.0	84.8	80.3	PR-Jan
A05-318019	14.0	19.0	16.5	34.0	25.5	29.8	38.5	26.5	32.5	26.3	PR-May§
A05-318020	20.0	16.0	18.0	13.5	30.0	21.8	29.0	42.5	35.8	25.2	PR-May
A05-318021	13.0	21.5	17.3	21.0	13.5	17.3	23.0	21.0	22.0	18.8	PR-May
A05-318025	22.0	36.0	29.0	33.0	22.5	27.8	30.0	44.5	37.3	31.3	PR-May
A05-318026	18.5	22.5	20.5	24.0	16.5	20.3	21.5	36.0	28.8	23.2	PR-May
A05-318031	28.5	22.0	25.3	25.0	25.0	25.0	34.0	29.0	31.5	27.3	PR-May
CX1834-1-6	19.0	16.0	17.5	23.0	15.5	19.3	27.5	21.0	24.3	20.3	PR-May

Table B1. Cont.

	Ames			Carlisle			Lewis				
Entry	Rep 1	Rep 2	Mean	Rep 3	Rep 4	Mean	Rep 5	Rep 6	Mean	Overall mean	Source
	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------
						%					
B01769B019	67.5	83.5	75.5	80.5	83.5	82.0	84.5	92.5	88.5	82.0	PR-May
Mean			56.5			53.2			64.8	58.2	
SE			6.9			9.1			5.6	7.3	
$LSD_{0.05}$			8.3			10.8			6.7	4.8	
$LSD_{0.01}$			11.3			14.7			9.1	6.5	

† Iowa 2005 harvest source

‡ Puerto Rico January 2007 harvest source

§ Puerto Rico May 2007 harvest source

APPENDIX C

CLIMATE DATA FOR THREE SEED PRODUCTION SOURCES FOR CHAPTER 1.

Table C1. Climate data for 30 days of seed fill for the Iowa 2005 harvest source¶.

Day	High temp	Average temp	Low temp	High humidity	Average humidity	Low humidity
	----------°C----------			----------%RH§----------		
30†	27.2	22.2	17.2	89.0	75.0	53.0
29	31.1	25.6	21.1	91.0	69.0	46.0
28	31.7	23.3	18.3	92.0	79.0	54.0
27	27.2	23.3	18.3	75.0	60.0	43.0
26	23.9	18.9	14.4	85.0	69.0	49.0
25	20.6	20.6	20.0	80.0	75.0	72.0
24	26.7	22.2	17.8	94.0	80.0	71.0
23	28.9	22.8	20.6	96.0	85.0	58.0
22	30.0	22.2	14.4	93.0	67.0	31.0
21	27.8	21.7	15.6	82.0	64.0	43.0
20	28.3	22.2	16.7	90.0	68.0	43.0
19	27.8	21.7	15.6	85.0	66.0	42.0
18	27.8	20.6	15.0	90.0	71.0	48.0
17	29.4	21.1	12.8	79.0	51.0	22.0
16	30.0	22.2	13.9	78.0	53.0	30.0
15	30.6	22.8	14.4	80.0	62.0	37.0
14	31.1	23.9	17.8	83.0	68.0	49.0
13	30.6	23.9	17.8	91.0	62.0	29.0
12	27.8	23.3	19.4	84.0	66.0	46.0
11	26.7	22.2	18.3	88.0	73.0	51.0
10	23.9	19.4	16.7	95.0	85.0	71.0
9	32.8	23.9	16.7	97.0	70.0	35.0
8	31.7	25.6	20.0	81.0	57.0	32.0
7	31.7	25.0	18.9	85.0	60.0	31.0
6	30.6	25.6	21.1	78.0	64.0	46.0
5	25.6	21.7	15.6	95.0	77.0	66.0
4	24.4	17.2	10.0	88.0	60.0	27.0
3	21.1	15.6	12.2	82.0	64.0	43.0
2	25.0	16.1	10.6	94.0	71.0	31.0
1‡	24.4	18.3	11.7	85.0	64.0	41.0
Mean	27.9	21.8	16.4	86.8	67.8	44.7

† 18 August 2005

‡ 18 September 2005, physiological maturity

§ % Relative humidity

¶ From www.wunderground.com/weatherstation/WXDailyHistory.asp?ID=KIABOONE3; accessed 20 June 2007

Table C2. Climate data for 30 days of seed fill for the Puerto Rico January harvest 2007 source¶.

Day	High temp	Average temp	Low temp	High humidity	Average humidity	Low humidity
	----------°C----------			----------%RH§----------		
30†	32.2	25.6	23.3	78.0	69.0	50.0
29	31.1	26.1	22.2	78.0	64.0	46.0
28	32.2	27.2	22.2	72.0	59.0	43.0
27	31.1	25.0	21.1	72.0	63.0	44.0
26	31.1	26.1	21.1	78.0	60.0	39.0
25	31.1	26.1	21.7	72.0	62.0	37.0
24	31.7	26.7	22.2	77.0	61.0	43.0
23	31.7	26.7	22.2	74.0	61.0	39.0
22	30.0	26.1	22.8	79.0	69.0	53.0
21	31.1	27.2	21.7	81.0	60.0	43.0
20	30.0	25.6	22.2	75.0	66.0	52.0
19	31.1	26.7	22.2	78.0	65.0	44.0
18	31.1	26.1	22.2	79.0	68.0	51.0
17	31.1	24.4	22.2	80.0	73.0	48.0
16	31.7	27.8	23.9	72.0	61.0	45.0
15	31.1	25.6	22.8	73.0	60.0	40.0
14	31.1	25.6	21.1	72.0	58.0	39.0
13	31.1	26.7	22.8	75.0	61.0	46.0
12	31.1	26.1	22.2	73.0	60.0	37.0
11	31.1	26.7	23.3	72.0	63.0	47.0
10	31.1	26.7	23.3	73.0	61.0	46.0
9	31.1	26.7	23.3	74.0	58.0	41.0
8	31.1	25.6	21.1	75.0	66.0	43.0
7	30.0	25.6	22.8	78.0	66.0	49.0
6	31.1	25.6	21.1	77.0	62.0	42.0
5	31.7	26.7	23.3	71.0	54.0	31.0
4	32.2	26.7	22.8	67.0	52.0	29.0
3	26.7	22.2	21.1	74.0	67.0	55.0
2	31.1	27.8	22.8	64.0	49.0	36.0
1‡	31.1	25.0	20.0	75.0	55.0	33.0
Mean	31.0	26.1	22.2	74.6	61.8	43.0

† 9 December 2006

‡ 7 January 2007, physiological maturity

§ % Relative humidity

¶ From http://www.wunderground.com/weatherstation/WXDailyHistory.asp?ID=KPRPONCE1; accessed 20 June 2007

Table C3. Climate data for 30 days of seed fill for the Puerto Rico May harvest 2007 source¶.

Day	High temp	Average temp	Low temp	High humidity	Average humidity	Low humidity
	----------°C----------			----------%RH§----------		
30†	31.7	26.1	22.8	87.0	68.0	43.0
29	27.2	24.4	22.8	89.0	85.0	69.0
28	31.1	25.6	23.3	87.0	77.0	53.0
27	31.7	26.1	22.8	87.0	75.0	52.0
26	30.0	24.4	21.7	88.0	78.0	50.0
25	31.1	27.2	19.4	73.0	48.0	38.0
24	31.7	25.6	20.0	75.0	50.0	30.0
23	31.7	25.6	19.4	70.0	49.0	32.0
22	31.1	26.1	20.0	70.0	49.0	33.0
21	31.7	27.2	20.6	63.0	49.0	40.0
20	33.9	30.6	26.7	68.0	53.0	40.0
19	33.3	28.3	22.8	74.0	57.0	35.0
18	32.8	28.9	24.4	69.0	54.0	41.0
17	33.9	28.9	25.0	71.0	56.0	36.0
16	33.9	28.9	24.4	73.0	57.0	39.0
15	33.3	27.8	23.3	73.0	58.0	37.0
14	33.3	27.8	22.2	69.0	59.0	42.0
13	33.9	28.9	23.9	70.0	58.0	40.0
12	33.3	28.3	23.3	77.0	61.0	43.0
11	31.7	27.8	25.0	72.0	64.0	50.0
10	33.9	28.9	23.9	80.0	65.0	47.0
9	34.4	29.4	25.6	85.0	68.0	46.0
8	32.2	27.8	24.4	88.0	76.0	54.0
7	33.3	28.3	23.9	82.0	67.0	51.0
6	32.2	27.8	25.6	75.0	57.0	38.0
5	31.1	27.2	24.4	78.0	63.0	45.0
4	33.3	27.8	22.2	80.0	59.0	33.0
3	33.3	27.2	23.3	73.0	63.0	46.0
2	33.3	28.3	22.8	79.0	59.0	39.0
1‡	33.3	28.9	25.6	64.0	54.0	38.0
Mean	32.4	27.5	23.2	76.3	61.2	42.7

† 30 March 2007

‡ 28 April 2007, physiological maturity

§ % Relative humidity

¶ From http://www.wunderground.com/weatherstation/WXDailyHistory.asp?ID=KPRPONCE1; accessed 20 June 2007

APPENDIX D

ANALYSES OF VARIANCE FOR FIELD EMERGENCE FOR CHAPTER 2

Table D1. Analysis of variances for field emergence of 43 of 48 progeny rows evaluated at two Iowa environments in 2006.

Sources of variation	df	Mean squares
Environment	1	86.8ns
Rep/environment	2	124.7
Genotype	42	2764.3**
Genotype x environment	42	134.1ns
Error	84	175.6
CV (%)		33.2

** significant at the 0.01 probability level
ns = not significant at the 0.05 probability level

Table D2. Analyses of variances for field emergence of 43 of 48 progeny rows evaluated at each Iowa environment in 2006.

		Mean squares	
Sources of variation	df	Ames	Burkey
Replication	2	57.4ns	192.0ns
Genotype	42	1476.6**	1421.9**
Error	84	204.7	146.6
CV (%)		36.5	29.8

** significant at the 0.01 probability level
ns = non-significant at the 0.05 probability level

APPENDIX E

MEAN PERFORMANCE OF EACH ENTRY FOR CHAPTER 2.

Table E1. Field emergence percentage of 31 $F_{2:3}$ lines and 17 parent and check lines grown at two Iowa environments in 2006.

		Ames			Burkey				
2006 Entry	2007 Entry	Rep 1	Rep 2	Mean	Rep 3	Rep 4	Mean	Overall mean	Population
		----------%----------							
760001	519001	25.0	40.0	32.5	45.0	10.0	27.5	30.0	Pop 4LS†
760002	519002	50.0	35.7	42.9	15.4	53.8	34.6	38.7	Pop 4LS
760003	519003	10.0	5.0	7.5	10.0	0.0	5.0	6.3	Pop 4LS
760004	519004	20.0	20.0	20.0	65.0	35.0	50.0	35.0	Pop 4LS
760005	519005	45.0	30.0	37.5	25.0	15.0	20.0	28.8	Pop 4LS
760006	519006	0.0	35.0	17.5	50.0	25.0	37.5	27.5	Pop 4LS
760007	519007	20.0	25.0	22.5	10.0	31.6	20.8	21.6	Pop 4LS
760008	Absent#	75.0	25.0	50.0	50.0	70.0	60.0	55.0	Pop 4LS
760009	Absent	35.0	20.0	27.5	20.0	35.0	27.5	27.5	Pop 4LS
760010	Absent	5.0	10.0	7.5	20.0	10.0	15.0	11.3	Pop 4LS
760011††	519008	5.0	Fill	5.0	Fill	Fill	5.0	5.0	Pop 4LS
760012††	519009	28.6	69.2	48.9	76.9	Fill	58.2	58.2	Pop 4LS
760013††	519010	8.3	0.0	4.2	0.0	Fill	0.0	2.8	Pop 4LS
760014††	Absent	0.0	0.0	0.0	Fill	Fill	0.0	0.0	Pop 4LS
760015	519011	0.0	20.0	10.0	5.0	0.0	2.5	6.3	Pop 4LS
760016	519012	10.0	20.0	15.0	10.0	5.0	7.5	11.3	Pop 5LS
760017	519013	7.1	46.2	26.6	46.2	15.4	30.8	28.7	Pop 5LS‡
760018	Absent	7.1	0.0	3.6	28.6	7.1	17.9	10.7	Pop 5LS
760019	Absent	35.3	5.9	20.6	11.8	5.9	8.8	14.7	Pop 5LS
760020††	Absent	0.0	30.0	15.0	40.0	Fill	23.3	23.3	Pop 5LS
760021	519014	57.1	35.7	46.4	46.2	30.8	38.5	42.4	Pop 2UL§
760022	519015	25.0	10.0	17.5	15.0	35.0	25.0	21.3	Pop 2UL
760023	Absent	75.0	60.0	67.5	60.0	70.0	65.0	66.3	Pop 2UL

Table E1. Cont.

2006 Entry	2007 Entry	Ames			Burkey			Overall mean	Population
		Rep 1	Rep 2	Mean	Rep 3	Rep 4	Mean		
		----------	----------	----------	%----------	----------	----------	----------	
760024	Absent	0.0	15.0	7.5	15.0	10.0	12.5	10.0	Pop 2UL
760025	519016	15.0	0.0	7.5	15.8	0.0	7.9	7.7	Pop 2UL
760026	Absent	0.0	0.0	0.0	16.7	8.3	12.5	6.3	Pop 2UL
760027	Absent	30.0	45.0	37.5	50.0	35.0	42.5	40.0	Pop 2UL
760028	Absent	33.3	45.5	39.4	9.1	27.3	18.2	28.8	Pop 3UL¶
760029	Absent	5.0	35.0	20.0	20.0	5.0	12.5	16.3	Pop 3UL
760030	Absent	66.7	64.7	65.7	82.4	88.2	85.3	75.5	Pop 3UL
760031	Absent	40.0	20.0	30.0	55.0	25.0	40.0	35.0	Pop 3UL
760032	519017	20.0	25.0	22.5	45.0	35.0	40.0	31.3	A04-314030
760033	519018	80.0	65.0	72.5	70.0	65.0	67.5	70.0	A04-642005
760034	519019	70.0	50.0	60.0	55.0	85.0	70.0	65.0	IA2069
760035	519020	80.0	85.0	82.5	65.0	75.0	70.0	76.3	IA2070
760036	Absent	20.0	5.0	12.5	15.0	10.0	12.5	12.5	A05-318011
760037	519021	20.0	10.0	15.0	25.0	20.0	22.5	18.8	A05-318020
760038	519059	70.0	80.0	75.0	90.0	85.0	87.5	81.3	B01769B019
760039	Absent	80.0	85.0	82.5	60.0	80.0	70.0	76.3	IA2064
760040	Absent	60.0	70.0	65.0	60.0	60.0	60.0	62.5	IA3017
760041	Absent	70.0	50.0	60.0	90.0	85.0	87.5	73.8	A04-218007
760042	519022	70.0	15.0	42.5	30.0	45.0	37.5	40.0	A04-442033
760043	519023	80.0	70.0	75.0	70.0	80.0	75.0	75.0	A05-318025
760044	519024	65.0	85.0	75.0	60.0	80.0	70.0	72.5	A04-542015
760045	Absent	75.0	85.0	80.0	80.0	80.0	80.0	80.0	IA2068
760046	Absent	80.0	95.0	87.5	85.0	85.0	85.0	86.3	IA1021
760047	Absent	90.0	80.0	85.0	80.0	75.0	77.5	81.3	IA3024

Table E1. Cont.

2006 Entry	2007 Entry	Ames			Burkey			Overall mean	Population
		Rep 1	Rep 2	Mean	Rep 3	Rep 4	Mean		
		----------%----------							
760048	519025	10.0	45.0	27.5	50.0	35.0	42.5	35.0	CX1834-1-6
Mean				38.5			40.3	39.6	
SE				14.3			12.1	13.3	
$LSD_{0.05}$				28.9			24.4	18.6	
$LSD_{0.01}$				38.6			32.7	24.7	

† A05-318011 x IA2070
‡ A05-318020 x IA2069
§ A05-218007 x A04-642005
¶ A05-314030 x A04-442033
# Not included in the 2007 field evaluation due to inadequate phytate levels, seed oil traits, or redundancy of pedigree.
†† Emergence percentages were not included in the calculation of the SE or LSD due to missing data.

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