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Genetic improvement of seedling emergence of soybean lines with low phytate

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Genetic improvement of seedling emergence of soybean lines with low phytate

by

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ABSTRACT

Seedling emergence of low-phytate (LP) soybean [*Glycine max* (L.) Merr.] lines has been reported to be lower than that of normal-phytate (NP) lines. One objective of this study was to evaluate if backcrossing the LP trait into a NP line would result in LP progeny with normal emergence. The LP line CX1834-1-6 (CX1834) was crossed to B01769B019 (B019), a NP line with reduced palmitate content, and three backcrosses were made to B019. A total of 36 BC_3F_4-derived LP lines from the population were evaluated at five locations in 2005 in comparison with CX1834, B019, and a NP cultivar IA3023. The mean phytate P and inorganic P content of all the backcross lines was not significantly different from CX1834. There were 18 backcross lines that had a mean field emergence that was significantly greater than CX1834 and not significantly different from B019. The results indicated that backcrossing seemed to be successful for developing LP lines with normal field emergence. A second objective of the study was to determine the effectiveness of warm germination, cold vigor, and accelerated aging tests for predicting field emergence of LP lines. Fifteen of the backcross lines were evaluated in the three tests that represented the range of field emergence that had been observed. The tests were useful for identifying lines with inferior field emergence, but were not reliable enough to replace field tests for identifying the best emerging lines.

INTRODUCTION

Monogastric animals are unable to effectively utilize the phytate P [*myo*-inositol 1,2,3,4,5,6-hexa*kis*phosphate] in soybean meal because they do not produce sufficient amounts of the enzyme phytase that is required for the breakdown of phytate (Erdman, 1979). Mutant lines were developed through chemical mutagenesis that contained ≈25% phytate P compared with ≈75% phytate P in normal soybean cultivars (Wilcox et al., 2000). Reducing phytate P and increasing inorganic P would increase the availability of P in the soybean meal fed to monogastric animals, reduce the amount of inorganic P added to their rations, and reduce the amount of P they excrete (Powers et al., 2006).

The impact of the LP trait on agronomic and seed traits of soybean was evaluated by Meis et al. (2003). They studied lines with the *mips* allele that controls reduced phytate and reduced raffinose saccharides. They found that the LP lines had significantly lower seedling emergence than NP lines. The emergence of LP lines was significantly influenced by the environment in which the seed was produced for planting. Seed produced in subtropical environments had significantly lower emergence than seed produced in a temperate environment.

Oltmans et al. (2005) compared LP and NP lines derived from crosses between the LP line CX1834 and three NP cultivars. The LP trait in CX1834 was controlled by the *pha1* and *pha2* alleles (Oltmans et al., 2004). The LP lines had a mean seedling emergence across three Iowa locations of 45% compared with 68% for the NP lines. They did not find a consistent significant difference between the two types of lines for other agronomic and seed traits. Hulke et al. (2004) evaluated LP lines with the *pha1* and *pha2* alleles in comparison with NP lines from a backcross population that was developed by crossing CX1834 to the NP

line B019 and backcrossing once to B019. They observed a mean seedling emergence of 65% for the LP lines and 87% for the NP lines averaged across three Iowa locations. Despite the lower emergence of the LP lines, their mean yield was not significantly different from the NP lines. They concluded that it should be possible to develop LP cultivars that yield as well as conventional cultivars, particularly if it is possible to minimize the reduction in seedling emergence of LP lines. One objective of our study was to evaluate if LP lines with normal seedling emergence could be obtained by incorporating the LP trait into a NP line through multiple backcrosses.

It would be desirable to be able to predict the seedling emergence of LP lines in the field through one or more laboratory tests. Meis et al. (2003) evaluated the effectiveness of four laboratory tests for predicting the field emergence of LP lines with the *mips* allele. They reported that the tetrazolium, warm germination, and cold vigor tests were not effective, but the accelerated aging test was useful for predicting field emergence. A second objective of our study was to evaluate the effectiveness of the warm germination, cold vigor, and accelerated aging tests for predicting the field emergence of LP lines with the *pha1* and *pha2* alleles.

LITERATURE REVIEW

Importance of the low-phytate trait

Soybean meal is an important source of protein in the feed rations of livestock. It also provides other important nutrients, including P. The seed of conventional soybean cultivars used to produce soybean meal contains about 4.3 g kg^{-1} phytate P and 0.7 g kg^{-1} inorganic P (Wilcox et al., 2000). The phytate P in soybean meal is unavailable to non-ruminant animals because they do not produce enough of the enzyme phytase that is required to break down phytate to an available form (Erdman, 1979). Undigested phytate P is excreted in the feces. When manure is applied to the soil, excessive amounts of P can become a source of water pollution (Daverede et al., 2004).

Phytase can be added to non-ruminant feed rations to improve the breakdown of phytate P to meet the dietary needs of non-ruminant animals (Adeola et al., 1995; Cromwell et al., 1993; Powers et al., 2006). Another means of increasing P availability in soybean meal is to develop cultivars with reduced phytate and increased inorganic P. Wilcox et al. (2000) used chemical mutagenesis to develop a mutant line with low phytate. The mutant line contained about 1.9 g kg^{-1} phytate P and 3.1 g kg^{-1} inorganic P, whereas the normal line contained about 4.3 g kg^{-1} phytate P and 0.7 g kg^{-1} inorganic P (Wilcox et al., 2000). They found that the reduction in phytate P was accompanied by an increase in inorganic P. The total P in the seed was the same as for NP cultivars (Oltmans et al., 2005).

Powers et al. (2006) evaluated four diets for swine that included NP soybean meal and no added phytase, NP soybean meal and phytase, LP soybean meal without any phytase, and LP soybean meal with added phytase. They concluded that the source of soybean meal did not significantly effect daily gain, feed intake, or feed efficiency. There was a significant

difference in P digestibility. The digestibility of P was 48.9% when pigs were fed LP diets compared to 42.4% when NP soybean meal was fed. Feeding LP soybean meal reduced fecal total P by 19% and water-soluble P by 17% compared with NP meal. They concluded that feeding LP soybean meal instead of NP soybean meal did not impact swine performance and that the reduction in fecal total P and water-soluble P could have a positive influence on the environment (Powers et al., 2006).

Genetic control of low-phytate trait

The source of LP used in my study was the line CX1834 developed by the USDA-ARS and Purdue University (Wilcox et al., 2000). The breeding line CX1515-4 was treated with ethyl methanesulfonate and the progeny were screened for increased inorganic P, which was used as an indirect indicator of reduced phytate. A LP phenotype was identified in two M2 plants: M153 and M766. The LP phenotype was confirmed in the M6 generation, indicating that the mutation was heritable and nonlethal. The breeding line CX1834 was selected from a cross between the mutant line M153-1-4-6-14 and the cultivar 'Athow' that has NP. CX1834 was selected from the population based on its yield potential.

Oltmans et al. (2004) evaluated the inheritance of the LP trait in a line derived from CX1834. They reported that the trait was controlled by two recessive alleles that exhibit duplicate dominant epistasis. The alleles were designated *pha1* and *pha2*. Only the *pha1pha1pha2pha2* genotype would give a LP phenotype.

Walker et al. (2006) identified two loci that control inorganic P levels in an F_2 population derived from the cross between 'AGS Boggs' and CX1834-1-2. CX1834-1-2 was a low-phytate line selected from the same population as the LP line studied by Oltmans

(2004). A locus on linkage group N accounted for 41% of the inorganic P variation, and a locus on linkage group L accounted for another 11%. The interaction of the two loci contributed another 8 to 11% of variation. They concluded that the two loci were the ones that had previously been identified as *pha1* and *pha2*.

Influence of low phytate on agronomic and seed traits

The first report on the performance of LP lines for agronomic and seed traits was by Meis et al. (2003). They studied lines with the *mips* allele that controls reduced phytate and raffinose saccharides content. They reported a significant reduction in seedling emergence of LP lines. The emergence of LP lines was significantly influenced by the environment in which the seed was produced. Seed produced in subtropical environments had significantly lower emergence than seed produced in a temperate environment.

Oltmans et al. (2005) evaluated three LP populations derived from crosses between CX1834 and three NP cultivars. $F_{2:4}$ lines were evaluated at three Iowa locations for seedling emergence and other agronomic and seed traits. They found a significant difference between the LP and NP lines for mean phytate P, inorganic P and other P, but there was not a significant difference in total P between the two types of lines. The LP lines had a mean seedling emergence of 45% compared with 68% for NP lines. They did not find significant difference in other agronomic and seed traits between the two types of lines.

Hulke et al. (2004) evaluated LP and NP lines from a backcross population that was developed by crossing CX1834 to B019. The BC_1F_1 seeds were obtained by crossing the F_1 plants back to B019. B019 was a line with NP and reduced palmitate developed jointly by Iowa State University and Pioneer Hi-Bred International, Inc. The reduced-palmitate trait in

B019 was controlled by the recessive alleles *fap1* and *fap3* (Fehr et al., 1991). A total of 20 BC_1F_2-derived lines that had LP and reduced-palmitate and 20 lines that had NP and reduced-palmitate were evaluated at three Iowa locations in 2003. The mean palmitate content of B019 was 34 g kg^{-1} compared with 117 g kg^{-1} for CX1834. They observed a mean seedling emergence of 65% for the LP lines and 87% for the NP lines. Despite the lower emergence of the LP lines, yield was not significantly different between the two types of lines. They concluded that it should be possible to develop LP cultivars that yield as well as conventional cultivars, particularly if it is possible to minimize the reduction in seedling emergence of LP lines.

Evaluation of seedling emergence of low-phytate lines

Meis et al. (2003) evaluated the effectiveness of four laboratory methods for predicting the seedling emergence of *mips* lines in the field. They reported that the tetrazolium test, warm germination, and cold vigor tests were not effective, but the accelerated aging test was effective in predicting field emergence.

MATERIALS AND METHODS

Line development

The lines used in our study were obtained from a backcross population that was developed by transferring the *pha1* and *pha2* alleles from CX1834 to B019. CX1834, the donor of the *pha1* and *pha2* alleles, was obtained from the USDA-ARS and Purdue University. B019 was a line homozygous for the *fap1* and *fap3* alleles for reduced palmitate that was developed jointly by Iowa State University and Pioneer Hi-Bred International, Inc (Fehr et al., 1991). The cross between B019 and CX1834 was made at the Agricultural Engineering and Agronomy Research Center near Ames, IA, in July 2001. The F_1 seeds and seeds of B019 were planted at the Iowa State University-University of Puerto Rico soybean-breeding nursery at Isabela, PR, in October 2001. To obtain suitable flowers for crossing, the F_1 plants were grown under artificial lights to extend the day length. The soil type at Isabela is a Coto clay (very-fine, kaolinite, isohyperthermic type Eutrustox). The F_1 plants were confirmed as hybrids using DNA marker analysis. The F_1 plants had the genotype *Pha1pha1Pha2pha2*. The F_1 plants were crossed to B019 and 36 BC_1F_1 seeds were obtained.

The BC_1F_1 seeds were planted at Isabela during February 2002 and the plants were harvested individually. The BC_1F_1 plants had one of four genotypes for the *pha* alleles: *Pha1Pha1Pha2Pha2, Pha1Pha1Pha2pha2, Pha1pha1Pha2Pha2*, or *Pha1pha1Pha2pha2*. The desired genotype was *Pha1pha1Pha2pha2* because it was the only one that could produce LP BC_1F_2 progeny. Eleven seeds from each BC_1F_1 plant were analyzed to identify those that had at least one LP seed, which indicated that the plant had the *Pha1pha1Pha2pha2* genotype. The technique used for phytate testing was a modification of the procedure described by Wilcox et al. (2000). A seed was crushed and placed in a 12 x 75

mm glass tube. An aliquot of 1 mL of 12.5% (w/v) trichloroacetic acid (TCA) and 25 m*M* magnesium chloride was added to the test tube to extract the inorganic P from the seed. A volume of 1 mL of Chen's reagent was added about 15 min after the TCA was added. Chen's reagent consisted of 1 vol 3 *M* sulfuric acid, 1 vol 0.02 *M* ammonium molybdate, 1 vol 10% (w/v) ascorbic acid, and 2 vol double-distilled water. The samples were allowed to stand for 15 min at room temperature. The solution became dark blue for an LP seed, but remained clear or became light blue for a NP seed.

The seeds from heterozygous BC_1F_1 plants were planted at Ames in 2002 and the second backcross was made by crossing B019 as the female parent to BC_1F_2 plants. The BC_1F_2 plants used for crossing were identified with a number. At maturity, the BC_2F_1 seed and each BC_1F_2 plant used as a male was harvested. To determine the genotype of the BC_1F_2 plant, one seed from each plant was tested for phytate content. If the seed had LP, three more seeds from that plant were tested for phytate. If the four seeds had LP, the BC_1F_2 plant was considered to have the genotype *pha1pha1pha2pha2* and the BC_2F_1 seeds that traced to that plant were saved.

The BC_2F_1 seeds were planted at Isabela, PR, during October 2002 and each plant was harvested individually. All the BC_2F_2 seeds from each BC_2F_1 plant were split with a razor blade into two pieces. About one-third of the seed was used for phytate testing and the remaining two-thirds with the embryonic axis was saved for planting. A total of 13 BC_2F_2 LP seeds and seeds of B019 were planted in January 2003 at Isabela and BC_3F_1 seeds were obtained.

All the BC_3F_1 seeds were planted in a greenhouse at Ames in 2003 and the seedlings were transplanted to the field at 6 plants m^{-1} in rows spaced 1.02 m apart. At maturity, the

BC_3F_1 plants were harvested and threshed individually. The BC_3F_2 seeds from each plant were split and the part without the embryonic axis was tested for phytate and fatty ester content. The method of fatty ester analysis by gas chromatography was described by Hammond (1991). The LP seeds with low saturates (palmitate + stearate) were planted in October 2003 at the Illinois Crop Improvement Association research station at Ponce, PR. The BC_3F_2 plants were harvested and threshed individually.

A five-seed bulk from each BC_3F_2 plant was tested for fatty ester content and those with low saturates had five individual seeds tested for phytate content. A total of 27 BC_3F_2 plants were selected that had low saturates and LP, and 50 seeds from each of the selected plants were planted during January 2004 at Ponce at 6 seeds m^{-1}. Two plots of 50 seeds each were planted of CX1834 and B019. The seedling emergence in each plot was determined by counting the number of plants that were harvested. All the BC_3F_3 plants from all the lines were harvested individually. For $BC_3F_{2:3}$ lines that had a seedling emergence of 75% or greater, five individual seeds from five plants were tested for phytate content. The $BC_3F_{2:3}$ lines that were homogenous for LP had five seeds tested from their remaining BC_3F_3 plants to identify all LP plants. A total of 285 plants that were homozygous for LP were selected for progeny testing.

The progeny test of the LP BC_3F_3 plants was grown at Ames during 2004. A 20-seed sample from each plant was retained for phytate testing and the remaining seeds were used to plant a two-row plot 2.74 m long at 20 seeds m^{-1}. Seedling emergence was determined when plants were in the V3 stage when there were three nodes on the main stem with fully developed leaves (Fehr and Caviness, 1977). The seedling emergence of CX1834 was 53% and B019 was 80%. Lines that had a seedling emergence of 75% or greater were selected for

phytate testing. For each of the 29 selected lines, one of the two rows was thinned to 18 plants to enhance seed production of each plant. To determine the inorganic P content of the selected lines, the 20 reserve seeds and 20 seeds of CX1834 and B019 were analyzed by Victor Raboy, USDA-ARS, Aberdeen, ID. The inorganic P contents were 3.58 g kg^{-1} for CX1834 and 0.19 g kg^{-1} for B019. For the 25 BC_3F_3-derived lines (families) with an inorganic P content of > 2.0 g kg^{-1} and 75% or greater field emergence, six BC_3F_4 plants were harvested from the row that had been thinned. For each of the six plants, five individual seeds were tested for phytate. All of the BC_3F_4 plants were homozygous for LP.

During October 2004, each of the 25 BC_3F_3- derived families were planted in an experiment at Ponce to evaluate seedling emergence and to obtain seed for subsequent tests. CX1834, B019, and IA3023 were included in the experiment. IA3023 was used as conventional NP cultivar with a maturity similar to that of the BC_3F_3-derived families. The experimental design was a randomized complete-block design with five replications. Each replication of the BC_3F_3-derived families was planted with 100 seeds from a different BC_3F_4 plant that traced to that family. Each plot was a single row 3.66 m long. The percentage of seedling emergence of all plots was determined 14 d after planting. The mean seedling emergence was 90% for CX1834, 91% for B019, and 87% for IA3023. The reduction in seedling emergence observed for CX1834 at Ames did not occur in this environment. Only the 18 BC_3F_3-derived families that had a mean seedling emergence of 86% or greater were selected for harvest. For each selected family, the two replications of their $BC_3F_{4:5}$ progeny with the highest emergence were threshed individually with a stationary bundle thresher. The plots of CX1834, B019, and IA3023 also were harvested. For each $BC_3F_{4:5}$ line, five

individual seeds were tested for phytate content and a five-seed bulk was tested for fatty ester content to confirm that they had LP and low saturates.

Field test

The 36 $BC_3F_{4:6}$ lines were evaluated at six locations for seedling emergence and at five locations for seedling emergence and agronomic and seed traits. The experiment had 40 entries, which included the 36 lines, two entries of CX1834 and one entry each of B019 and IA3023. All of the locations used for the experiment were planted with the seed harvested from the October 2004 planting at Ponce. A randomized complete-block design with two replications was used for each location.

The first field test was planted at Ponce on 28 January 2005. The soil type is a San Antón sandy clay loam (fine-loamy, mixed, superactive, isohyperthermic Cumulic Haplustoll). For each entry, 200 seeds were planted in a single row 7.62 m long. Seedling emergence was determined 21 d after planting. Yield, maturity, lodging, and plant height were not measured because growth of plants at Ponce is not representative of that in Iowa. At maturity, each plot was harvested in bulk with a stationary bundle thresher.

In the summer of 2005, the experiment was planted at Ames, Carlisle, Lewis, Osceola, and Ottumwa, IA. The soil type at Carlisle is a Tama silty clay loam (fine-silty, mixed, superactive, mesic Typic Agriudoll), at Ames is a Nicolett loam (fine-loamy, mixed, mesic Aquic Hapludoll), at Lewis is a Marshall silty clay loam (fine-silty, mixed, mesic Typic Hapludoll), at Osceola is a Grundy silty clay loam (fine, montmorillonitic, mesic Aquic Argiudoll), and at Ottumwa is a Coppock silt loam (fine-silty, mixed, mesic Mollic Ochraqualf). 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 2 May at Ames, 3 May at Carlisle, 4 May at Ottumwa, 6 May at Lewis, and 10 May at Osceola.

Data were collected on all plots at all locations for seedling emergence, plant height, lodging, seed yield, seed size, and protein, oil, and, fatty ester content. Maturity was recorded at Ames and Carlisle. Seedling emergence was determined by counting the number of plants in each plot at the V3 stage. Maturity was recorded as days after 31 August when 95% of the pods in a plot had reached their mature color. Plant height was measured as the distance from the soil surface to the terminal node on the main stem. Lodging was determined at maturity on a scale of 1 (all plants erect) to 5 (all plants prostrate). The plots were harvested with a self-propelled plot combine (Almaco, Nevada, IA) at all of the locations, except Ames. A stationary bundle thresher was used at Ames to avoid any seed mixture among plots. The weight and moisture content of the seed was determined and seed yield was expressed on a 13%-moisture basis. Seed size was obtained by weighing 200 random whole seeds from each plot. Protein, oil, and moisture were measured on a 300-gram sample with a near-infrared transmission spectrometer (Tecator AB, Hooganas, Sweden). Protein and oil content was determined on a 13%-moisture basis. Fatty ester content was measured on a five-seed bulk sample by gas chromatography.

Phytate P and inorganic P tests

Phytate P and inorganic P content were analyzed in duplicate with the seed harvested from the two replications at Ames in 2005. A sample of 100 seeds was ground to pass through a 1-mm screen using a UDY Cyclone sample mill (UDY Corporation, Fort Collins,

CO). Phytate P was determined by capillary zone electrophoresis (CZE) as described by Nardi et al. (1992) and refined by Joel D. Nott of the Protein Facility at Iowa State University. A 20 mg sample of ground seed was weighed, placed in a scintillation vial, and extracted in 10 mL of 0.5 m*M* L-aspartic acid. The solution was stirred at room temperature for 20 min with 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).

CZE was performed with a fused silica FS-175 capillary (31.2 cm X 75 µM i.d., capillary length to detector of 21.0 cm) obtained from Upchurch Scientific (Oak Harbor, WA). The chemicals used were benzoic acid, phytic acid, phosphoric acid and hexadecyltrimethylammonium bromide (CTAB) from Sigma-Aldrich (St. Louis, MO) and L-aspartic acid, L-histidine, potassium phosphate, sodium hydroxide, and hydrochloric acid from Fisher Scientific (Fair Lawn, NJ).

CZE was run using reverse polarity (detector side was positive) at ambient temperatures with indirect UV detection at 254 nm. To overcome the electroosmotic flow, a 1.0 m*M* solution of CTAB was used to coat the capillary before running the samples (Janini et al., 1993). The running buffer/background electrolyte used was 50 m*M* benzoic acid adjusted to pH 6.3 with 90 m*M* of L-histidine (Schöppenthau et al, 1996). The run conditions for the CZE were as follows. The capillary was rinsed with 1 m*M* CTAB for 1 min at 20 psi followed by a 1 min rinse at 20 psi with running buffer. Samples were injected using reverse polarity at 7kV for 5 sec. The ions were separated using reverse polarity at

14 kV for 5 min. Detection of the ions was done indirectly at 254 nm by reversing the polarity of the detector. After every 20 injections, the capillary was regenerated by rinsing it with 1 N hydrochloric acid for 5 min, distilled water for 2 min, 0.1 N sodium hydroxide for 10 min, distilled water for 3 min, 1 m*M* CTAB for 5 min, and background electrolyte for 5 min. All rinses were done at 20 psi.

The internal calibrant used for CZE was 0.5 m*M* L-aspartic acid. From the CZE data, a ratio of phytic acid to L-aspartic acid was determined by dividing the corrected peak area for phytic acid by the corrected peak area for L-aspartic acid. Standards of phytic acid (0 m*M* to 0.020 m*M*) were run and a standard curve of phytic acid/L-aspartic acid was generated. From this curve, the phytic acid of all the test samples was determined.

The method used to quantify inorganic P was a modification of the technique described by Chen et al. (1956). A 0.5 g sample of ground seed was extracted in 20 mL of 12% trichlorocetic acid that contained 0.2 *M* magnesium chloride. The samples were stirred overnight at 4° C. Following extraction, the samples were centrifuged for 20 min. A volume of 100 µL of the aqueous solution was added to 3.9 µL of double-distilled water and 4 mL of Chen's reagent was added to the solution. Samples were allowed to stand for 2 hr at room temperature and were analyzed at 820 nm on a Varian Cary 50 Bio UV-Visable spectrophotometer (Palo Alto, CA).

Germination tests

The lines used to evaluate the effectiveness of laboratory germination tests for predicting field emergence included the eight BC_3F_4-derived lines with a mean field emergence equal to or greater than B019 at the five Iowa locations, seven BC_3F_4-derived

lines with lower field emergence than B019, two entries of CX1834, and one entry each of B019 and IA3023. The 19 entries were evaluated with a warm germination, cold vigor, and accelerated aging test at the Iowa State University Seed Science Center. Each test was conducted with two replications of 100 seeds for each entry in a randomized complete-block design. Each replication of a test was conducted in a separate germination cart. The seed used for the study was from one replication of the entries harvested at Ames in 2005.

The warm germination test was conducted by planting each entry on a fiberglass food service tray that measured 45 x 66 cm. Two sheets of 19-ply Kimpak® (Neenah, WI) were moistened with 825 mL of water and placed on a tray (AOSA, 2004). The 100 seeds of four entries were planted on top of the Kimpak and the trays were placed in a germination cart. The germination cart was 0.5 m wide x 0.7 m deep x 1.6 m high. The cart was made of aluminum, except for the back that was made of Plexiglas™ to allow light penetration. Each cart was placed in a growth room at 25° C for 7 d. The evaluation of seeds for germination was based on standards provided by the Association of Official Seed Analysts (AOSA, 2005).

For the cold test, one sheet of 19-ply Kimpak® was placed on a tray, moistened with 1,100 mL of water, and placed in a growth room overnight at 10° C. The following morning, the 100 seeds of each of four entries were planted on a tray. After planting, each tray was covered with a mixture of 1 soil: 4 sand to a depth 2.54 cm and placed in a germination cart. The carts were placed in a growth room at 10° C for 7 d, after which the carts were moved to a growth room at 25° C for 7 d (AOSA, 1983). Germination percentages were determined based on standards provided by the Association of Official Seed Analysts (AOSA, 2005).

The accelerated aging test began by placing 42 g of seed from each entry in a wire basket. The wire basket was placed over 40 mL of distilled water in an acrylic box and covered. The boxes were placed in a chamber at 41° C for 72 h (AOSA, 2005). After 72 h, the 100 seeds of each of four entries were planted on two-layers of Kimpak® that had been moistened with 825 mL of water and placed on a tray. After planting, each tray was covered with 13 mm of moist sand and placed in a germination cart. The carts were put in a growth room at 25° C for 7 d, after which germination was evaluated as described by the Association of Official Seed Analysts (AOSA, 2005).

DATA ANALYSIS

The data for each trait at the Iowa locations were analyzed as a randomized complete-block design by the linear model procedure of the SAS statistical software (release 8.02) (SAS Institute, 2001). The field emergence in Puerto Rico was 85% for CX1834, 74% for B019, and 92% for IA3023. The normal emergence of CX1834 prevented any meaningful assessment of differences in field emergence among the backcross lines in that environment and the data were not included in the analysis of variance.

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

$$Y_{ijk} = \mu + E_i + RP/E_{j/i} + G_k + EG_{ik} + e_{ijk},$$

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 ith environment,

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

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

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

e_{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. An F-test was used to determine significance of main effects. The environment X main effect interactions were used to test the main effects across environments.

Table 1. Analysis of variance and expected means squares across five Iowa environments in 2005.

Sources of variation	Degrees of freedom	df	Expected mean squares
Environments (E)	e-1	4	$\sigma^2 + g\sigma^2_{RP/E} + rg\sigma^2_E$
Replications/E (RP/E)	(rp-1)e	5	$\sigma^2 + g\sigma^2_{RP/E}$
Genotypes (G)	g-1	39	$\sigma^2 + r\sigma^2_{GE} + re\Phi_G$
G x E	(g-1)(e-1)	156	$\sigma^2 + r\sigma^2_{GE}$
Error	e(rp-1)(g-1)	195	σ^2
Total	erg-1	399	

The linear additive model for agronomic and seed traits at each Iowa environment was:

$$Y_{ij} = \mu + RP_i + G_j + e_{ij},$$

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,

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

Replications were considered random effects and genotypes were considered fixed effects. An F-test was used to determine the significance of the main effects. The error mean squares were used to test significance of genotype effects.

Table 2. Analysis of variance and expected means squares for each Iowa environment in 2005.

Sources of variation	Degrees of freedom	df	Expected mean squares
Replications (RP)	rp-1	1	$\sigma^2 + g\sigma^2_{RP}$
Genotypes (G)	g-1	39	$\sigma^2 + r\Phi_G$
Error	(r-1)(g-1)	39	σ^2
Total	rg-1	79	

The data for phytate P and inorganic P were analyzed as a randomized complete-block design by the linear model procedure of the SAS statistical software (release 8.02) (SAS Institute, 2001). Only LP lines were included in the analysis of variance to determine if there was significant difference among them.

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

$$Y_{ij} = \mu + RP_i + G_j + e_{ij},$$

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

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

Replications were considered random effects and genotypes were considered fixed effects. An F-test was used to determine the significance of the main effects. The error mean squares were used to test significance of genotype effects.

Table 3. Analysis of variance and expected means squares for phytate P and inorganic P.

Sources of variation	Degrees of freedom	df	Expected mean squares
Replications (RP)	rp-1	1	$\sigma^2 + g\sigma^2_{RP}$
Genotypes (G)	g-1	37	$\sigma^2 + r\Phi_G$
Error	(r-1)(g-1)	37	σ^2
Total	rg-1	76	

The data for each germination test were analyzed as a randomized complete-block design by the linear model procedure of the SAS statistical software (release 8.02) (SAS Institute, 2001).

The linear additive model for each germination test was:

$$Y_{ij} = \mu + RP_i + G_j + e_{ij},$$

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,

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

Replications were considered random effects and genotypes were considered fixed effects. An F-test was used to determine significance of main effects. The error mean squares were used to test significance of genotype effects.

Table 4. Analysis of variance and expected means squares for the three germination tests.

Sources of variation	Degrees of freedom	df	Expected mean squares
Replications (RP)	rp-1	1	$\sigma^2 + g\sigma^2_{RP}$
Genotypes (G)	g-1	18	$\sigma^2 + r\Phi_G$
Error	(r-1)(g-1)	18	σ^2
Total	rg-1	37	

Phenotypic correlations among traits were based on entry means across environments and computed using the (CORR) procedure of the SAS statistical software (release 8.02) (SAS Institute, 2001)

A least significant difference (LSD) was calculated to determine differences between genotypes at the 0.05 and 0.01 probability levels. The standard error of the mean (SEM) and the coefficient of variation (CV) also were calculated as follows:

$$LSD = t_\alpha \sqrt{2MSE / n}$$

$$SEM = \sqrt{MSE / n}$$

$$CV\ (\%) = [\sqrt{MSE} / Mean] \times 100$$

where:

t = critical t value at either the 0.05 or 0.01 probability level,

MSE = the error mean squares for an individual environment or genotype x environment interaction for the combined analysis,

n = number of observations in each entry mean, and

Mean = mean of all entries for each trait.

RESULTS AND DISCUSSION

The mean phytate P and inorganic P contents of the backcross lines and the LP parent CX1834 were not significantly different from each other, but were significantly different from the recurrent parent B019 and the cultivar IA3023 (Table 5). The mean phytate P content of the backcross lines was 143 mg g^{-1} x 10^{-3} compared with 185 mg g^{-1} x 10^{-3} for CX1834 and 878 mg g^{-1} x 10^{-3} for B019. The mean inorganic P content was 3.02 mg g^{-1} for the backcross lines, 3.10 mg g^{-1} for CX1834, and 0.27 mg g^{-1} for B019 (Table 5). The inverse relationship between phytate P and inorganic P was consistent with the results of Wilcox et al. (2000) and Oltmans et al. (2005).

CX1834 had significantly lower field emergence in Iowa than B019 and IA3023 (Table 5). The mean seedling emergence of CX1834 was 54% averaged across the five locations, which was the same as its mean emergence of 54% reported by Hulke et al. (2004). There were significant differences among the backcross lines for field emergence with a range of 47% to 75%. There were 18 of the 36 backcross lines that had significantly greater field emergence than CX1834 and that were not significantly different than B019. The results indicated that the reduced field emergence of LP lines reported by Hulke et al. (2004) and Oltmans et al. (2005) may have been overcome in some of the backcross lines and that the reduced emergence of CX1834 may be due, at least in part, to factors other than its reduced phytate P and elevated inorganic P contents. Additional research will be required to determine if the improved emergence of the best backcross lines is repeatable over a broad range of environments with seed produced in subtropical and temperate climates and if the improved emergence is heritable.

Warm germination, cold vigor, and accelerated aging tests were conducted to determine their effectiveness in predicting the field emergence of LP lines. All of the tests were effective in predicting the reduced emergence of CX1834. The reduced germination of the line was associated with seed decay due to infection of seed storage fungi. The same infection was not observed for B019 and IA3023 when planted on the same germination trays. Seeds of backcross lines that failed to germinate also were infected by seed storage fungi. The susceptibility of CX1834 to infection by seed storage fungi may account in part for its reduced field emergence.

The three tests differed in the mean germination percentages of the backcross lines and their phenotypic correlations with field emergence (Table 5). The backcross lines had a mean germination of 79% in the warm germination test, 72% in the cold vigor test, and 65% in the accelerated aging test. The phenotypic correlation of field emergence with the warm germination test of 0.49 was not significant ($P>0.05$), but the correlation of 0.82 for the cold vigor test and 0.66 for the accelerated aging test was significant ($P<0.01$). Of the four lines with <60% field emergence, two were among the five lines with the lowest percentages in the warm germination test, all of them were included in the poorest five lines for the cold vigor test, and two of them were the poorest in the accelerated aging test. The three tests were equal for identifying the lines with the best field emergence. Of the four lines with >70% field emergence, only two of them were among the best lines in each of the three tests. The results indicated that either the cold vigor or accelerated aging tests could be used to discard inferior lines, but neither test could replace field evaluation for identifying the lines with the best emergence.

There were 34 of the 36 backcross lines not significantly different than B019 in seed yield. Of the eight lines with field emergence equal or better than B019, none of them were significantly different than B019 in yield. This result supported the conclusion of Hulke et al. (2004) that the LP trait per se when controlled by the *pha* alleles does not adversely affect seed yield.

The majority of the backcross lines were not significantly different than B019 in maturity, lodging, height, seed weight, protein content, and oil content (Table 5). The results indicated that the LP trait should not adversely affect the development of cultivars comparable to conventional ones for those traits.

The majority of the backcross lines had significantly higher saturates than B019 (Table 1). The increased saturates were due to greater palmitate and stearate in the backcross lines than in B019, which can be attributed to the significantly greater content of the two fatty acids in CX1834. The saturate content of the oil is critical if the intent is to label it as low in saturated fat in accordance with requirements of the U.S. Food and Drug Administration. A liquid oil must have <1.25 g in a 14 g serving (<89 g kg^{-1}) to be designated as a low-saturate oil (U.S. Food and Drug Administration, 1999). Soybean oil contains ≈10 g kg^{-1} of saturated fatty acids other than palmitate and stearate (Hulke et al., 2004). Consequently, the content of palmitate and stearate should not exceed 79 g kg-1 and preferably should be less than 75 g kg^{-1} to take into account possible environment effects on fatty acid content and the possibility of co-mingling with conventional soybeans during commercial production. There were 13 of the backcross lines that had 75 g kg^{-1} or less saturates. This was an improvement over the LP lines evaluated by Hulke et al. (2004) that had a mean of 83 g kg^{-1} saturates, with the best line containing 78 g kg^{-1} saturates. Our

results indicated that it should be possible to develop LP lines with acceptable saturate content. The low-saturate lines used for crossing in the breeding program should contain as little palmitate + stearate as possible to maximize the frequency of acceptable segregates.

There was significant variation among the backcross lines for oleate, linoleate, and linolenate content (Table 5). It should be possible to develop LP cultivars that are similar to low-saturate cultivars for these fatty acids.

Table 5. Mean agronomic and seed performance of lines evaluated at five Iowa environments and germination percentages in three laboratory tests.

Entry	Phytate P†	Inorganic P†	Field Emergence	Warm germination	Cold vigor	Accelerated aging	Yield	Maturity	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates‡
	mg g^{-1} x 10^{-3}	mg g^{-1}	%	%	%	%	kg ha^{-1}	d§	score¶	cm	mg sd^{-1}	g kg^{-1}#	g kg^{-1}#	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318004	125	3.10	62	69	71	59	2889	16	1.9	86	141	343	193	40	40	293	567	60	80
318011	135	3.09	71	81	82	61	3041	18	1.9	81	144	349	189	38	35	276	586	65	74
318013	115	3.19	47	70	55	51	2793	19	2.0	81	142	349	189	37	36	283	579	65	73
318014	191	3.30	53	80	66	43	2770	17	1.9	86	145	352	187	37	36	273	589	65	73
318015	187	3.42	54	80	60	62	2845	20	2.0	86	142	346	192	38	39	284	574	64	77
318019	134	2.86	70	77	78	78	2957	17	2.0	86	146	342	194	38	37	283	579	62	76
318020	145	2.83	75	78	85	77	3135	17	2.1	89	149	343	193	37	37	283	577	65	75
318021	169	2.81	70	89	80	71	2996	16	2.0	87	147	341	196	39	38	285	575	64	77
318022	137	3.21	60	77	60	61	3021	20	2.2	89	146	340	193	38	37	272	588	66	74
318024	140	2.65	69	88	81	79	3059	18	1.9	87	147	342	194	38	37	288	574	63	75
318025	151	3.04	75	79	72	69	3122	17	2.0	88	147	341	193	39	38	281	578	65	77
318026	208	2.92	70	83	81	69	2990	16	2.1	84	142	340	194	38	38	282	578	65	75
318028	105	2.84	65	78	74	63	3010	18	2.1	87	148	341	194	38	39	297	566	61	76

Table 5. Continued

Entry	Phytate P†	Inorganic P†	Field Emergence	Warm germination	Cold vigor	Accelerated aging	Yield	Maturity	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates‡
	mg g^{-1} x 10^{-3}	mg g^{-1}	%	%	%	%	kg ha^{-1}	d§	score¶	cm	mg sd^{-1}	g kg^{-1}#	g kg^{-1}#	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318029	143	2.85	57	76	70	70	2948	18	2.1	85	147	338	196	38	38	276	581	66	77
318031	141	2.76	75	83	73	61	2974	16	2.0	88	145	343	194	37	38	285	575	66	74
CX1834	185	3.10	54	44	45	41	2316	14	1.6	61	160	353	188	114	51	242	524	69	165
B019	878	0.27	69	87	86	88	2996	19	2.1	90	142	347	194	36	34	277	584	69	70
IA3023	962	0.29	75	93	80	87	3470	21	1.7	82	143	335	199	101	45	271	522	61	146
LSD 0.05	117	1	7	9	8	8	258	3	0.2	5	6	6	3	3	2	19	18	4	3

† = Mean of two replications for seed harvested at Ames, IA, 2005.

‡ = Palmitate + stearate.

§ = Days after 31 August.

¶ = Score of 1 (all plants erect) to 5 (all plants prostrate).

= Expressed on a 13 g kg^{-1}-moisture basis.

APPENDIX A

ANALYSIS OF VARIANCE FOR AGRONOMIC AND SEED TRAITS ACROSS ENVIRONMENTS

Table A1. Analysis of variance for agronomic and seed traits including backcross lines, parents, and checks across five Iowa environments in 2005.

		Mean squares						
Sources of variation†	df	Emergence (%)	Yield (kg ha^{-1})	Lodging (score‡)	Height (cm)	Seed weight (mg sd^{-1})	Protein (g kg^{-1})	Oil (g kg^{-1})
E	4	10558.5**	30475616.8**	6.5**	9151.7**	8190.3**	16364.9**	3595.0**
RP/E	5	378.8**	342269.9**	0.1ns	71.5**	225.4**	229.9**	66.7**
G	39	565.9**	411432.7**	0.2**	372.8**	201.5**	234.2**	86.0**
G x E	156	69.8**	85519.9ns	0.1ns	30.3**	46.1**	45.4**	14.3**
Error	195	32.1	73072.5	0.1	18.3	30.6	26.6	7.6
CV (%)		9.1	9.3	12.0	5.0	3.8	1.5	1.4

		Mean squares					
Sources of variation	df	Palmitate (g kg^{-1})	Stearate (g kg^{-1})	Oleate (g kg^{-1})	Linoleate (g kg^{-1})	Linolenate (g kg^{-1})	Saturates§ (g kg^{-1})
E	4	244.1*	298.8**	89730.9**	58039.3**	5022.6**	278.8ns
RP/E	5	34.5**	21.3**	384.5ns	251.2ns	70.9**	91.4**
G	39	3689.6**	113.3**	1465.7**	2521.3**	42.9**	5006.3**
G x E	156	9.7**	6.0**	484.2*	417.5*	16.6ns	14.0ns
Error	195	6.6	4.0	340.9	297.0	14.1	11.0
CV (%)		6.0	5.2	6.6	3.0	5.9	4.1

* significant at the 0.05 probability level.
** significant at the 0.01 probability level.
ns = not significant at the 0.05 probability level.
† = E = environments, RP/E = replications within environments, G = genotypes.
‡ = score 1 (all plants erect) to 5 (all plants prostrate).
§ = palmitate + stearate.

APPENDIX B

ANALYSIS OF VARIANCE FOR AGRONOMIC AND SEED TRAITS AT INDIVIDUAL ENVIRONMENTS

Table B1. Analysis of variance for agronomic and seed traits including backcross lines, parents, and checks at Ames, IA, in 2005.

		Mean squares						
Sources of variation†	df	Emergence (%)	Yield (kg ha^{-1})	Lodging (score‡)	Height (cm)	Seed weight (mg sd^{-1})	Protein (g kg^{-1})	Oil (g kg^{-1})
RP	1	181.7*	3859.6ns	0.1ns	140.5*	41.3ns	30.0ns	18.1ns
G	39	253.7**	326621.0**	0.1ns	152.3**	55.9**	84.6**	28.6**
Error	39	42.5	121030.6	0.0	26.0	19.0	30.9	12.4
CV (%)		14.9	16.7	10.5	6.3	3.1	1.6	1.9

		Mean squares					
Sources of variation	df	Palmitate (g kg^{-1})	Stearate (g kg^{-1})	Oleate (g kg^{-1})	Linoleate (g kg^{-1})	Linolenate (g kg^{-1})	Saturates§ (g kg^{-1})
RP	1	4.1ns	23.9*	261.0ns	200.7ns	23.9ns	8.3ns
G	39	685.5**	41.3**	664.8ns	1001.8*	34.5*	1001.9**
Error	39	10.0	4.7	574.0	497.3	16.9	12.2
CV (%)		7.4	5.4	8.8	3.9	5.7	4.2

* significant at the 0.05 probability level.
** significant at the 0.01 probability level.
ns = not significant at the 0.05 probability level.
† = RP = replications, G = genotypes.
‡ = score 1 (all plants erect) to 5 (all plants prostrate).
§ = palmitate + stearate.

Table B2. Analysis of variance for agronomic and seed traits including backcross lines, parents, and checks at Carlisle, IA, in 2005.

		Mean squares						
Sources of variation†	df	Emergence (%)	Yield (kg ha^{-1})	Lodging (score‡)	Height (cm)	Seed weight (mg sd^{-1})	Protein (g kg^{-1})	Oil (g kg^{-1})
RP	1	743.5**	326028.0**	0.4*	67.8*	705.6**	165.3*	18.1ns
G	39	102.0**	118167.0**	0.2**	81.6**	57.2**	78.1**	23.1**
Error	39	18.8	39434.7	0.1	11.9	17.5	27.6	5.5
CV (%)		6.1	5.2	10.1	3.4	2.9	1.6	1.2

		Mean squares					
Sources of variation	df	Palmitate (g kg^{-1})	Stearate (g kg^{-1})	Oleate (g kg^{-1})	Linoleate (g kg^{-1})	Linolenate (g kg^{-1})	Saturates§ (g kg^{-1})
RP	1	2.9ns	0.1ns	30.5ns	73.3ns	158.8**	1.9ns
G	39	812.9**	18.3**	333.2*	750.4**	16.4*	1034.3**
Error	39	8.3	3.1	159.1	166.5	9.3	13.0
CV (%)		6.4	4.8	4.9	2.2	4.7	4.4

* significant at the 0.05 probability level.
** significant at the 0.01 probability level.
ns = not significant at the 0.05 probability level.
† = RP = replications, G = genotypes.
‡ = score 1 (all plants erect) to 5 (all plants prostrate).
§ = palmitate + stearate.

Table B3. Analysis of variance for agronomic and seed traits including backcross lines, parents, and checks at Lewis, IA, in 2005.

		Mean squares						
Sources of variation†	df	Emergence (%)	Yield (kg ha^{-1})	Lodging (score‡)	Height (cm)	Seed weight (mg sd^{-1})	Protein (g kg^{-1})	Oil (g kg^{-1})
RP	1	25.9ns	48722.9ns	0.0ns	9.8ns	0.0ns	162.5**	43.5**
G	39	104.1**	91976.9*	0.0ns	88.0**	36.2*	66.9**	20.4**
Error	39	31.3	48443.0	0.0	10.8	17.8	15.3	4.1
CV (%)		8.1	7.5	11.2	4.2	3.1	1.1	1.0

		Mean squares					
Sources of variation	df	Palmitate (g kg^{-1})	Stearate (g kg^{-1})	Oleate (g kg^{-1})	Linoleate (g kg^{-1})	Linolenate (g kg^{-1})	Saturates§ (g kg^{-1})
RP	1	85.6**	5.4ns	777.1ns	351.6ns	6.8ns	132.9**
G	39	846.4**	18.0**	486.6**	706.7**	17.5ns	1066.2**
Error	39	2.2	3.5	224.0	176.7	11.5	5.4
CV (%)		3.3	4.7	5.5	2.3	5.1	2.8

* significant at the 0.05 probability level.
** significant at the 0.01 probability level.
ns = not significant at the 0.05 probability level.
† = RP = replications, G = genotypes.
‡ = score 1 (all plants erect) to 5 (all plants prostrate).
§ = palmitate + stearate.

Table B4. Analysis of variance for agronomic and seed traits including backcross lines, parents, and checks at Osceola, IA, in 2005.

		Mean squares						
Sources of variation†	df	Emergence (%)	Yield (kg ha^{-1})	Lodging (score‡)	Height (cm)	Seed weight (mg sd^{-1})	Protein (g kg^{-1})	Oil (g kg^{-1})
RP	1	61.3ns	42660.5ns	0.0ns	4.0ns	0.1ns	65.9ns	39.4**
G	39	69.7**	100924.4*	0.1**	83.6**	123.7**	107.2**	35.2**
Error	39	30.8	52281.4	0.0	18.6	16.4	18.2	4.1
CV (%)		8.2	7.9	10.3	5.7	2.7	1.2	1.0

		Mean squares					
Sources of variation	df	Palmitate (g kg^{-1})	Stearate (g kg^{-1})	Oleate (g kg^{-1})	Linoleate (g kg^{-1})	Linolenate (g kg^{-1})	Saturates§ (g kg^{-1})
RP	1	13.7ns	5.5ns	523.4ns	535.7ns	38.6ns	36.6ns
G	39	622.2**	32.9**	353.5ns	1074.2**	21.4ns	918.4**
Error	39	7.1	3.2	214.2	211.4	16.8	13.1
CV (%)		6.2	5.0	5.5	2.5	6.3	4.6

* significant at the 0.05 probability level.
** significant at the 0.01 probability level.
ns = not significant at the 0.05 probability level.
† = RP = replications, G = genotypes.
‡ = score 1 (all plants erect) to 5 (all plants prostrate).
§ = palmitate + stearate.

Table B5. Analysis of variance for agronomic and seed traits including backcross lines, parents, and checks at Ottumwa, IA, in 2005.

		Mean squares						
Sources of variation†	df	Emergence (%)	Yield (kg ha^{-1})	Lodging (score‡)	Height (cm)	Seed weight (mg sd^{-1})	Protein (g kg^{-1})	Oil (g kg^{-1})
RP	1	881.5**	1290078.3**	0.2ns	135.6*	379.9*	726.0**	214.5**
G	39	315.8**	115823.0ns	0.1ns	88.7**	113.0ns	79.1*	35.7**
Error	39	36.9	104172.8	0.1	24.1	82.3	40.8	12.0
CV (%)		10.6	11.4	16.3	5.6	5.6	1.8	1.8

		Mean squares					
Sources of variation	df	Palmitate (g kg^{-1})	Stearate (g kg^{-1})	Oleate (g kg^{-1})	Linoleate (g kg^{-1})	Linolenate (g kg^{-1})	Saturates§ (g kg^{-1})
RP	1	66.2**	71.8**	330.5ns	94.8ns	126.5**	277.1**
G	39	761.3**	27.0**	1564.3**	658.1ns	19.4ns	1041.8**
Error	39	5.2	5.4	533.2	433.4	16.2	11.3
CV (%)		5.6	5.8	6.8	3.9	7.9	4.2

* significant at the 0.05 probability level.
** significant at the 0.01 probability level.
ns = not significant at the 0.05 probability level.
† = RP = replications, G = genotypes.
‡ = score 1 (all plants erect) to 5 (all plants prostrate).
§ = palmitate + stearate.

APPENDIX C

ANALYSIS OF VARIANCE FOR MATURITY

Table C1. Analysis of variance for maturity including backcross lines, parents, and checks across two Iowa environments in 2005.

Sources of variation†	df	Mean squares
		Maturity (days‡)
E	1	5569.6**
RP/E	2	21.1**
G	39	10.7**
G x E	39	4.3*
Error	78	2.4
CV (%)		8.8

* significant at the 0.05 probability level.
** significant at the 0.01 probability level.
† = E = environments, RP/E = replications within environments, G = genotypes.
‡ = days after 31 August.

Table C2. Analysis of variance for maturity including backcross lines, parents, and checks for individual environments in 2005.

Sources of variation†	df	Mean squares	
		Maturity (days‡)	
		Ames	Carlisle
RP	1	33.8**	8.5ns
G	39	3.7ns	11.2**
Error	39	2.4	2.4
CV (%)		6.5	13.3

** significant at the 0.01 probability level.
ns = not significant at the 0.05 probability level.
† = RP = replications, G = genotypes.
‡ = days after 31 August.

APPENDIX D

ANALYSIS OF VARIANCE FOR PHYTATE P AND INORGANIC P

Table D1. Analysis of variance for phosphorous composition of LP lines at Ames, IA, in 2005.

Sources of variation†	df	Mean squares	
		Phytate P (mg g^{-1} x 10^{-3})	Inorganic P (mg g^{-1})
RP	1	15780.23**	0.00ns
G	37	1118.24ns	0.07ns
Error	37	1919.70	0.08
CV (%)		30.3	9.4

** significant at the 0.01 probability level.
ns = not significant at the 0.05 probability level.
† = RP = replications, G = genotypes.

APPENDIX E

ANALYSIS OF VARIANCE FOR WARM GERMINATION, COLD VIGOR, AND ACCELERATED AGING TESTS

Table E1. Analysis of variance for seedling performance of selected lines for laboratory tests.

Sources of variation†	df	Mean squares		
		Warm germination (%)	Cold vigor (%)	Accelerated aging (%)
RP	1	168.42**	0.03ns	8.53ns
G	18	341.74**	318.33**	389.80**
Error	18	17.42	13.47	15.80
CV (%)		5.5	5.2	6.2

** significant at the 0.01 probability level.
ns = not significant at the 0.05 probability level.
† = RP = replications, G = genotypes.

APPENDIX F

MEAN PERFORMANCE OF EACH ENTRY ACROSS ENVIRONMENTS

Table F1. Mean performance for agronomic and seed traits of all lines across five environments in Iowa in 2005.

Entry	Phytate P†	Inorganic P†	Field Emergence	Yield	Maturity	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates‡
	mg g^{-1} x 10^{-3}	mg g^{-1}	%	kg ha^{-1}	days§	score¶	cm	mg sd^{-1}	g kg^{-1}#	g kg^{-1}#	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318001	117	3.10	63	2871	18	2.0	87	153	345	194	37	38	296	565	64	75
318002	134	2.95	63	3023	18	2.1	89	152	341	196	39	36	281	581	63	75
318003	137	3.23	63	2997	19	2.2	86	153	345	193	37	37	285	576	65	75
318004	125	3.10	62	2889	16	1.9	86	141	343	193	40	40	293	567	60	80
318005	112	3.06	52	2779	19	2.1	89	150	342	194	38	38	282	578	64	76
318006	127	3.16	56	2874	18	2.0	83	145	343	194	37	37	281	582	63	74
318007	132	2.99	53	2634	18	2.2	89	144	344	194	38	39	276	583	64	76
318008	152	3.25	67	3124	16	2.2	89	151	344	193	38	38	272	588	64	76
318009	128	3.06	50	2554	18	2.0	81	144	356	185	38	37	310	555	62	74
318010	139	3.12	68	3027	16	1.9	83	145	354	187	38	35	291	572	64	73
318011	135	3.09	71	3041	18	1.9	81	144	349	189	38	35	276	586	65	74
318012	112	3.03	57	2855	19	2.1	85	146	348	189	37	37	282	579	64	75
318013	115	3.19	47	2793	19	2.0	81	142	349	189	37	36	283	579	65	73
318014	191	3.30	53	2770	17	1.9	86	145	352	187	37	36	273	589	65	73
318015	187	3.42	54	2845	20	2.0	86	142	346	192	38	39	284	574	64	77
318016	158	2.78	61	2941	16	2.0	87	147	341	195	38	38	295	568	61	75
318017	139	2.81	67	2882	17	2.1	89	142	338	196	38	39	292	570	61	77
318018	148	3.01	62	2936	22	2.1	90	145	345	192	38	38	288	573	63	76
318019	134	2.86	70	2957	17	2.0	86	146	342	194	38	37	283	579	62	76
318020	145	2.83	75	3135	17	2.1	89	149	343	193	37	37	283	577	65	75
318021	169	2.81	70	2996	16	2.0	87	147	341	196	39	38	285	575	64	77
318022	137	3.21	60	3021	20	2.2	89	146	340	193	38	37	272	588	66	74
318023	148	3.16	60	2942	18	2.1	85	148	341	195	37	38	293	570	62	75
318024	140	2.65	69	3059	18	1.9	87	147	342	194	38	37	288	574	63	75

Table F1. Continued.

Entry	Phytate P†	Inorganic P†	Field Emergence	Yield	Maturity	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates‡
	mg g^{-1} x 10^{-3}	mg g^{-1}	%	kg ha^{-1}	days§	score¶	cm	mg sd^{-1}	g kg^{-1}#	g kg^{-1}#	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318025	151	3.04	75	3122	17	2.0	88	147	341	193	39	38	281	578	65	77
318026	208	2.92	70	2990	16	2.1	84	142	340	194	38	38	282	578	65	75
318027	153	3.05	60	2923	18	2.1	88	147	341	194	38	39	284	578	62	76
318028	105	2.84	65	3010	18	2.1	87	148	341	194	38	39	297	566	61	76
318029	143	2.85	57	2948	18	2.1	85	147	338	196	38	38	276	581	66	77
318030	138	3.27	63	2987	18	2.0	86	150	342	194	38	37	274	588	63	75
318031	141	2.76	75	2974	16	2.0	88	145	343	194	37	38	285	575	66	74
318032	136	2.86	55	2971	18	2.1	89	144	339	196	38	38	286	576	63	75
318033	124	2.89	63	2842	18	2.0	84	142	342	194	38	38	273	586	65	76
318034	141	2.74	58	2958	18	2.1	87	146	339	196	37	39	284	578	62	76
318035	155	2.97	58	3075	19	2.1	90	147	340	195	38	37	282	580	63	75
318036	174	3.32	53	2816	20	2.1	87	141	339	194	39	37	285	575	63	77
CX1834	185	3.10	54	2316	14	1.6	61	160	353	188	114	51	242	524	69	165
B019	878	0.27	69	2996	19	2.1	90	142	347	194	36	34	277	584	69	70
IA3023	962	0.29	75	3470	21	1.7	82	143	335	199	101	45	271	522	61	146
SEM	40	0.2	3	93	1	0.1	2	2	2	1	1	1	7	6	1	1
LSD 0.05	117	1	7	258	3	0.2	5	6	6	3	3	2	19	18	4	3
LSD 0.01	157	1	10	341	4	0.3	6	8	8	4	4	3	26	24	5	4

† = Mean of two replications for seed harvested at Ames, IA, in 2005.

‡ = Palmitate + stearate.

§ = Days after 31 August.

¶ = Score of 1 (all plants erect) to 5 (all plants prostrate).

= Expressed on a 13 g kg^{-1}-moisture basis.

APPENDIX G

MEAN PERFORMANCE OF EACH ENTRY AT INDIVIDUAL ENVIRONMENTS

Table G1. Mean performance for agronomic and seed traits of all lines at Ames, IA, in 2005.

Entry	Phytate P	Inorganic P	Field emergence	Yield	Maturity	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates†
	mg g^{-1} x 10^{-3}	mg g^{-1}	%	kg ha^{-1}	days‡	score§	cm	mg sd^{-1}	g kg^{-1}¶	g kg^{-1}¶	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318001	117	3.10	41	2182	24	2.0	86	148	357	187	36	40	315	534	75	76
318002	134	2.95	39	2228	23	2.0	89	146	351	190	38	39	280	569	74	77
318003	137	3.23	36	2322	25	2.3	86	142	356	184	37	37	276	576	74	74
318004	125	3.10	38	1973	23	2.0	83	134	356	184	49	44	283	557	67	93
318005	112	3.06	44	1946	25	2.3	85	141	362	182	36	42	283	566	74	78
318006	127	3.16	38	1877	23	2.0	79	137	356	185	36	41	271	576	76	76
318007	132	2.99	40	2035	24	2.5	83	139	359	185	38	43	258	586	75	81
318008	152	3.25	56	2658	22	2.5	91	149	355	188	38	39	261	589	73	77
318009	128	3.06	30	1407	25	2.0	79	135	366	179	37	39	308	546	70	76
318010	139	3.12	51	2265	21	2.0	75	133	363	181	36	36	283	571	74	72
318011	135	3.09	49	2272	23	2.0	76	133	355	185	37	37	263	591	72	74
318012	112	3.03	42	1857	25	2.3	82	141	361	181	36	39	306	551	68	75
318013	115	3.19	26	1878	24	2.0	78	135	357	184	37	37	278	578	71	73
318014	191	3.30	24	1659	23	2.0	78	127	360	180	37	36	249	601	77	73
318015	187	3.42	39	2058	26	2.0	83	137	359	183	38	39	274	575	75	76
318016	158	2.78	38	1949	22	2.3	80	140	350	190	38	41	292	560	69	79
318017	139	2.81	51	2394	25	2.0	90	136	358	185	37	42	297	561	64	78
318018	148	3.01	33	2045	27	2.3	85	141	355	184	38	38	281	571	72	76
318019	134	2.86	59	2475	23	2.3	87	143	349	190	39	36	249	607	69	75
318020	145	2.83	60	2406	23	2.0	89	141	352	187	38	38	267	585	73	76
318021	169	2.81	48	2286	22	2.0	83	136	348	191	38	44	295	556	68	82
318022	137	3.21	39	2089	27	2.3	82	134	354	182	36	35	252	597	81	71
318023	148	3.16	44	2125	24	2.3	87	136	351	188	37	40	272	576	75	76
318024	140	2.65	49	2127	23	2.0	86	138	349	188	38	37	266	587	72	75

Table G1. Continued.

Entry	Phytate P	Inorganic P	Field emergence	Yield	Maturity	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates†
	mg g^{-1} x 10^{-3}	mg g^{-1}	%	kg ha^{-1}	days‡	score§	cm	mg sd^{-1}	g kg^{-1}¶	g kg^{-1}¶	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318025	151	3.04	67	2578	23	2.3	88	137	354	187	40	38	267	584	71	78
318026	208	2.92	46	2199	24	2.0	82	135	350	188	39	40	255	588	78	79
318027	153	3.05	35	1564	24	2.3	80	137	354	183	37	41	288	566	68	78
318028	105	2.84	40	2069	23	2.3	85	140	350	189	38	41	292	563	67	79
318029	143	2.85	44	2151	23	2.0	80	141	351	188	38	39	256	590	76	77
318030	138	3.27	38	1985	23	2.0	77	143	346	191	40	39	248	596	77	79
318031	141	2.76	69	2621	23	2.3	91	145	352	189	36	37	274	576	76	73
318032	136	2.86	37	2011	23	2.0	82	133	349	189	37	40	272	580	71	77
318033	124	2.89	48	2194	24	2.0	81	139	352	187	38	38	279	579	66	76
318034	141	2.74	41	2092	24	2.3	79	136	351	188	36	40	259	593	71	77
318035	155	2.97	43	2565	25	2.3	87	143	353	187	39	36	253	599	72	76
318036	174	3.32	35	1639	25	2.0	75	130	352	186	37	39	296	559	70	76
CX1834	185	3.10	33	916	22	1.9	49	149	374	177	109	57	245	510	80	166
B019	878	0.27	61	2247	24	2.0	84	131	358	187	34	35	278	573	80	69
IA3023	962	0.29	73	2876	26	1.8	83	137	344	193	102	43	255	533	67	145
SEM	31	0.2	5	245	1	0.2	4	4	4	2	2	2	17	16	3	2
LSD 0.05	117	1	13	704	3	0.4	10	9	11	7	6	4	48	45	8	7
LSD 0.01	155	1	18	942	4	0.6	14	12	15	10	9	6	65	60	11	9

† = Palmitate + stearate.

‡ = Days after 31 August.

§ = Score of 1 (all plants erect) to 5 (all plants prostrate).

¶ = Expressed on a 13 g kg^{-1}-moisture basis.

Table G2. Mean performance for agronomic and seed traits of all lines at Carlisle, IA, in 2005.

Entry	Field emergence	Yield	Maturity	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates†
	%	kg ha^{-1}	days‡	score§	cm	mg sd^{-1}	g kg^{-1}¶	g kg^{-1}¶	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318001	75	3811	12	2.3	108	152	321	204	37	35	259	606	64	72
318002	72	3940	13	2.5	105	150	319	205	46	35	253	603	64	81
318003	73	3802	13	2.5	105	153	325	202	39	35	261	597	68	74
318004	77	3663	10	2.3	97	135	314	206	40	38	278	583	62	77
318005	63	3753	12	2.5	107	154	320	204	39	37	268	591	65	76
318006	63	3978	13	2.3	103	146	321	204	40	35	253	612	61	74
318007	64	3325	13	2.8	110	145	323	203	38	35	272	593	62	73
318008	78	3747	11	2.5	102	144	323	201	39	35	245	620	62	73
318009	61	3644	11	2.5	100	142	325	201	40	34	276	586	63	74
318010	76	3792	12	2.5	103	147	334	197	39	35	258	606	61	74
318011	81	4043	12	2.3	103	151	328	199	41	34	259	599	67	74
318012	63	3962	14	2.5	104	143	323	201	41	35	248	613	63	76
318013	62	3715	14	2.5	99	143	330	199	37	35	255	609	64	73
318014	69	3784	11	2.0	104	149	328	198	40	37	277	585	62	77
318015	62	3498	15	2.3	105	133	318	205	39	40	260	596	65	79
318016	74	3853	10	2.5	102	147	314	205	40	39	276	582	63	79
318017	74	3848	10	2.8	105	139	308	208	41	36	256	603	64	77
318018	69	3754	17	2.3	109	144	325	200	39	37	262	597	63	77
318019	77	3858	10	2.3	98	144	314	205	41	37	273	586	65	77
318020	84	3890	11	2.8	105	146	319	203	40	35	247	609	70	75
318021	83	3839	11	2.5	107	144	316	204	39	36	257	600	68	75
318022	68	4066	14	2.5	104	146	319	204	38	37	274	595	56	75
318023	70	3675	11	2.5	103	146	313	206	40	36	260	600	64	76
318024	75	3878	14	2.3	102	148	323	203	38	35	267	595	64	74

Table G2. Continued.

Entry	Field emergence	Yield	Maturity	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates†
	%	kg ha^{-1}	days‡	score§	cm	mg sd^{-1}	g kg^{-1}¶	g kg^{-1}¶	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318025	83	4083	12	2.5	103	149	316	204	41	35	256	601	67	76
318026	83	3930	7	2.5	98	140	311	207	41	37	256	600	67	77
318027	70	3881	11	2.5	107	148	317	205	39	35	257	608	61	74
318028	74	3879	13	2.5	103	148	320	204	40	36	259	603	63	76
318029	65	3814	13	2.5	107	144	312	207	41	37	265	593	65	78
318030	71	3990	13	2.5	107	151	319	204	39	33	236	628	65	71
318031	81	4019	9	2.5	103	145	316	204	38	37	253	609	64	75
318032	69	3935	13	2.5	105	146	312	207	39	37	283	579	62	76
318033	81	3866	12	2.5	108	143	317	205	37	37	261	599	66	74
318034	68	3818	13	2.3	104	149	318	206	40	39	273	582	66	79
318035	66	3904	13	2.3	105	144	321	203	39	35	252	611	64	73
318036	61	3658	14	2.5	107	139	317	205	48	35	250	605	63	83
CX1834	61	3189	5	1.5	79	159	334	193	118	46	228	537	71	164
B019	73	3787	14	2.3	107	141	322	207	34	30	254	618	64	64
IA3023	74	4636	16	1.8	100	145	315	206	107	43	239	547	64	150
SEM	3	140	1	0.2	2	3	4	2	2	1	9	9	2	3
LSD 0.05	9	402	3	0.5	7	8	11	5	6	4	26	26	6	7
LSD 0.01	12	538	4	0.7	9	11	14	6	8	5	34	35	8	10

† = Palmitate + stearate.

‡ = Days after 31 August.

§ = Score of 1 (all plants erect) to 5 (all plants prostrate).

¶ = Expressed on a 13 g kg^{-1}-moisture basis.

Table G3. Mean performance for agronomic and seed traits of all lines at Osceola, IA, in 2005.

Entry	Field emergence	Yield	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates†
	%	kg ha^{-1}	score‡	cm	mg sd^{-1}	g kg^{-1}§	g kg^{-1}§	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318001	69	2671	1.8	70	154	346	195	38	38	265	591	67	76
318002	69	2917	1.8	80	151	341	198	37	34	265	602	63	71
318003	66	2824	2.0	72	152	344	198	37	36	268	591	67	74
318004	72	2963	1.5	75	144	344	195	39	35	262	603	62	73
318005	61	2913	1.5	75	154	341	197	39	37	247	611	66	76
318006	69	3024	1.8	72	145	345	195	38	35	262	601	64	73
318007	57	2573	1.5	76	142	345	196	38	37	263	599	64	74
318008	69	3177	1.8	90	149	344	195	38	35	253	605	68	74
318009	59	2478	1.5	72	150	369	182	39	36	293	568	64	75
318010	69	2999	1.5	74	143	362	185	37	32	293	574	64	69
318011	76	2924	1.5	70	144	359	187	37	33	280	583	67	70
318012	63	2711	1.5	72	143	352	188	37	35	249	608	70	72
318013	59	2690	1.5	72	145	354	188	37	34	269	594	67	70
318014	67	2902	1.5	81	145	350	189	36	31	244	626	63	67
318015	61	2655	1.5	71	144	348	192	38	35	262	599	65	73
318016	71	3119	1.5	80	151	336	200	36	33	271	604	56	69
318017	73	2625	1.8	77	145	342	199	38	36	283	580	62	75
318018	74	3276	1.5	83	144	343	194	39	33	255	607	66	72
318019	72	2826	1.5	77	145	347	196	38	34	271	593	64	72
318020	77	3223	2.0	84	149	339	198	37	35	261	602	66	72
318021	72	2800	1.5	75	146	344	197	40	35	269	592	64	75
318022	73	2946	2.0	83	151	345	191	38	34	242	615	71	72
318023	71	2875	1.5	75	147	341	198	38	33	269	598	63	71
318024	68	3147	1.5	79	148	341	196	38	36	263	597	66	74

Table G3. Continued.

Entry	Field emergence	Yield	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates†
	%	kg ha^{-1}	score‡	cm	mg sd^{-1}	g kg^{-1}§	g kg^{-1}§	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318025	71	3033	1.5	79	150	340	196	39	38	259	598	66	77
318026	78	3022	1.5	74	145	341	198	38	34	254	608	66	72
318027	66	3031	1.5	79	150	341	197	40	38	269	587	66	79
318028	72	3190	1.8	77	146	340	197	37	37	283	581	62	74
318029	66	3255	1.5	79	153	334	199	40	37	258	594	72	76
318030	67	2885	1.5	80	149	341	197	39	36	261	602	62	75
318031	75	3037	1.5	86	147	345	196	38	35	260	598	69	73
318032	60	2846	1.8	81	141	342	196	37	34	263	599	66	72
318033	61	2549	1.5	67	139	349	194	37	37	263	596	67	74
318034	66	2820	1.5	79	144	335	199	38	36	276	592	59	73
318035	63	2970	1.5	84	151	339	197	37	35	281	582	65	73
318036	57	3130	1.8	81	148	335	198	39	34	247	610	70	73
CX1834	60	2506	1.4	58	179	353	191	110	51	246	525	69	161
B019	73	3154	2.0	80	143	350	193	41	35	270	586	68	76
IA3023	76	3198	1.5	74	151	341	199	90	44	293	512	61	134
SEM	4	162	0.1	3	3	3	1	2	1	10	10	3	3
LSD 0.05	11	462	0.3	9	8	9	4	5	4	30	29	8	7
LSD 0.01	15	619	0.4	12	11	12	5	7	5	40	39	11	10

† = Palmitate + stearate.

‡ = Score of 1 (all plants erect) to 5 (all plants prostrate).

§ = Expressed on a 13 g kg^{-1}-moisture basis.

Table G4. Mean performance for agronomic and seed traits of all lines at Lewis, IA, in 2005.

Entry	Field emergence	Yield	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates†
	%	kg ha^{-1}	score‡	cm	mg sd^{-1}	g kg^{-1}§	g kg^{-1}§	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318001	60	3006	2.0	80	144	348	195	39	37	279	579	66	76
318002	73	3215	2.0	80	146	348	194	37	36	271	592	65	73
318003	78	2895	2.0	80	141	347	192	39	39	276	581	65	78
318004	72	3101	2.0	86	138	343	196	40	41	281	576	62	81
318005	53	3085	2.0	88	142	336	197	41	39	262	591	67	80
318006	71	2894	1.8	76	137	342	196	37	37	266	594	65	75
318007	56	2514	1.8	77	138	346	194	39	41	268	586	67	80
318008	71	3016	2.0	77	132	338	197	38	40	277	577	67	79
318009	58	2752	2.0	74	138	353	189	37	37	306	559	61	74
318010	78	3102	1.8	79	131	352	187	40	37	273	582	68	77
318011	73	3065	2.0	77	132	342	193	39	36	257	602	66	76
318012	69	2870	1.8	79	139	343	193	39	39	253	599	70	79
318013	58	2508	1.8	70	130	347	190	37	38	257	598	69	76
318014	66	2920	1.8	76	148	357	186	38	38	278	579	67	76
318015	58	2850	2.0	76	136	344	194	39	43	295	562	63	81
318016	70	2871	1.8	83	137	347	193	40	38	271	585	66	78
318017	72	2934	2.0	80	131	338	196	39	40	275	581	66	79
318018	73	2794	2.0	83	140	346	193	40	41	272	583	64	81
318019	76	2943	2.0	77	135	337	196	39	39	289	570	63	78
318020	78	3178	1.8	80	142	341	195	39	39	264	587	71	78
318021	75	3009	1.8	80	140	344	195	39	39	283	575	64	78
318022	67	2969	2.0	83	135	339	195	39	39	277	578	67	78
318023	66	2908	1.8	75	139	341	195	39	39	288	572	63	78
318024	77	3227	2.0	77	138	342	194	39	38	267	593	64	76

Table G4. Continued.

Entry	Field emergence	Yield	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates†
	%	kg ha^{-1}	score‡	cm	mg sd^{-1}	g kg^{-1}§	g kg^{-1}§	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318025	77	2919	2.0	81	135	341	194	39	40	302	557	63	78
318026	81	3153	2.0	80	137	346	194	37	38	272	588	66	75
318027	76	3031	2.0	83	133	336	198	38	41	264	590	67	79
318028	76	3167	1.8	85	140	346	192	39	38	271	587	65	77
318029	65	2810	2.0	71	136	339	196	38	39	269	588	66	77
318030	74	3140	2.0	80	138	342	194	38	38	260	600	63	77
318031	73	2841	2.0	74	135	344	195	39	39	264	588	71	77
318032	67	3142	1.8	83	132	341	197	39	39	274	586	62	78
318033	71	2960	1.8	81	135	347	192	39	38	250	603	69	77
318034	73	3167	2.0	83	141	348	194	38	38	270	585	69	77
318035	72	3003	2.0	80	135	335	198	37	42	299	559	63	78
318036	66	3042	2.0	89	138	342	196	39	38	272	581	69	78
CX1834	58	2379	1.5	56	144	353	188	120	49	231	532	68	169
B019	68	3029	2.0	80	137	353	194	36	34	262	592	76	70
IA3023	75	3279	1.8	71	135	330	202	107	44	267	519	63	151
SEM	4	156	0.1	2	3	3	1	1	1	11	9	2	2
LSD 0.05	11	445	0.4	7	9	8	4	3	4	30	27	7	5
LSD 0.01	15	596	0.6	9	11	11	5	4	5	41	36	9	6

† = Palmitate + stearate.

‡ = Score of 1 (all plants erect) to 5 (all plants prostrate).

§ = Expressed on a 13 g kg^{-1}-moisture basis.

Table G5. Mean performance for agronomic and seed traits of all lines at Ottumwa, IA, in 2005.

Entry	Field emergence	Yield	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates†
	%	kg ha^{-1}	score‡	cm	mg sd^{-1}	g kg^{-1}§	g kg^{-1}§	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318001	71	2685	2.0	89	167	352	191	35	41	361	515	49	76
318002	62	2817	2.0	89	169	348	192	35	36	337	542	50	71
318003	61	3144	2.0	86	179	354	191	35	39	344	533	48	74
318004	52	2743	1.8	90	155	357	186	33	41	359	518	49	74
318005	40	2197	2.3	90	160	349	191	34	38	348	531	50	72
318006	37	2598	2.0	83	161	351	190	33	37	355	526	49	70
318007	49	2722	2.3	100	156	349	193	36	37	322	551	55	73
318008	64	3021	2.3	84	180	362	185	36	40	323	549	53	76
318009	42	2492	2.0	80	155	367	177	35	37	365	513	50	72
318010	68	2975	1.8	84	172	362	184	36	37	349	525	53	73
318011	73	2904	1.8	81	161	361	185	37	38	319	554	53	75
318012	47	2877	2.3	89	163	362	185	33	38	356	523	51	71
318013	32	3174	2.3	86	156	360	185	37	38	356	516	53	74
318014	38	2585	2.0	89	157	367	181	36	37	317	555	56	72
318015	51	3165	2.3	93	159	361	186	36	41	330	538	55	78
318016	54	2915	2.0	93	163	358	186	34	38	367	511	49	73
318017	64	2610	2.0	90	159	346	193	35	40	350	526	49	75
318018	59	2814	2.3	90	159	356	187	34	40	368	508	50	74
318019	64	2681	2.0	89	163	363	183	35	41	334	540	51	75
318020	77	2979	2.0	89	167	363	184	34	41	375	502	48	74
318021	70	3045	2.0	89	170	353	191	37	38	319	552	53	75
318022	52	3036	2.3	91	164	344	195	37	39	315	555	54	76
318023	49	3126	2.3	88	174	358	189	33	41	373	507	46	74
318024	74	2918	1.8	90	165	357	188	34	41	378	499	48	75

Table G5. Continued.

Entry	Field emergence	Yield	Lodging	Height	Seed weight	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates†
	%	kg ha^{-1}	score‡	cm	mg sd^{-1}	g kg^{-1}§	g kg^{-1}§	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318025	76	2998	1.8	89	166	357	188	37	39	321	549	56	75
318026	61	2644	2.3	89	152	355	186	35	39	372	506	49	74
318027	53	3105	2.0	93	169	357	188	34	38	341	537	50	72
318028	63	2744	2.0	86	168	350	191	35	41	378	499	47	76
318029	46	2707	2.5	89	159	354	191	36	39	334	541	50	75
318030	67	2937	2.0	86	170	363	185	36	40	364	512	49	75
318031	77	2353	1.8	86	153	360	185	32	40	373	505	50	72
318032	42	2923	2.3	91	166	352	191	36	37	336	538	52	74
318033	56	2638	2.0	84	153	346	192	38	43	313	551	55	81
318034	43	2892	2.3	91	160	346	195	34	40	343	538	46	74
318035	48	2931	2.3	91	162	351	191	38	39	323	548	52	77
318036	49	2611	2.0	85	151	352	189	34	41	362	518	45	75
CX1834	55	2589	1.6	65	170	350	193	112	52	263	518	55	164
B019	73	2764	2.0	98	159	352	193	38	35	321	550	56	73
IA3023	78	3363	1.5	80	147	344	195	100	50	300	501	48	150
SEM	4	228	0.2	3	6	5	2	2	2	16	15	3	2
LSD 0.05	12	653	0.7	10	18	13	7	5	5	47	42	8	7
LSD 0.01	16	874	0.9	13	25	17	9	6	6	63	56	11	9

† = Palmitate + stearate.

‡ = Score of 1 (all plants erect) to 5 (all plants prostrate).

§ = Expressed on a 13 g kg^{-1}-moisture basis.

Table G6. Mean performance for agronomic and seed traits of all lines at Ponce, PR, for the January 2005 planting.

Entry	Field emergence	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates§
	%	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318001	81	296	223	43	33	270	593	61	76
318002	87	283	225	46	33	282	579	60	79
318003	83	281	227	45	34	257	599	64	80
318004	77	281	227	46	34	289	572	59	80
318005	73	289	226	44	33	276	586	61	77
318006	79	286	224	46	35	272	584	63	81
318007	61	301	220	42	34	276	585	63	76
318008	74	302	220	47	34	277	583	60	80
318009	68	294	218	47	36	276	578	63	83
318010	89	291	219	50	36	263	587	64	86
318011	90	302	218	47	33	266	590	64	80
318012	78	291	218	47	36	261	590	65	84
318013	82	300	214	46	34	273	584	63	80
318014	81	308	210	46	35	280	577	63	80
318015	83	288	225	45	35	322	541	58	80
318016	79	287	223	49	35	274	579	62	84
318017	81	285	226	49	37	260	591	63	86
318018	81	290	227	48	34	271	584	63	82
318019	82	286	222	48	35	261	591	65	83
318020	88	280	227	48	34	285	574	60	82
318021	84	296	220	47	37	276	579	62	83
318022	86	292	220	50	34	274	579	63	84
318023	72	281	229	49	38	280	572	62	87
318024	81	286	227	47	38	271	580	64	85

Table G6. Continued.

Entry	Field emergence	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates†
	%	g kg^{-1}‡	g kg^{-1}‡	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
318025	86	298	219	46	34	295	566	59	80
318026	86	292	223	47	36	289	566	61	84
318027	74	298	221	46	34	279	582	60	79
318028	82	296	221	47	33	278	578	64	80
318029	73	287	223	48	34	291	567	60	82
318030	75	304	217	46	33	294	567	59	79
318031	85	292	221	45	35	279	580	61	80
318032	80	291	228	45	34	267	588	66	79
318033	83	290	223	47	35	287	570	62	82
318034	78	296	220	46	35	270	585	64	81
318035	71	290	224	45	35	291	571	59	79
318036	76	292	225	45	36	268	588	63	81
CX1834	85	309	215	130	49	211	541	69	179
B019	74	293	229	42	33	306	558	61	75
IA3023	92	297	225	115	38	229	552	66	152

† = Palmitate + stearate.

‡ = Expressed on a 13 g kg^{-1}-moisture basis.

APPENDIX H

PHENOTYPIC CORRELATIONS BETWEEN AGRONOMIC AND SEED TRAITS

Table H1. Phenotypic correlation coefficients among all entries and backcross entries for agronomic and seed traits.

Trait	Lines§	Inorganic P	Emergence	Yield	Maturity	Lodging	Height	Seed size	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates
Phytate P	All	-0.94**	0.32*	0.35*	0.23	-0.30	-0.03	-0.19	-0.14	0.27	0.38*	0.13	-0.20	-0.34*	0.12	0.34*
(mg g^{-1} x 10^{-3})	BC	0.17	0.11	0.14	-0.13	-0.10	0.10	-0.25	-0.09	0.03	0.14	-0.01	-0.20	0.17	0.18	0.07
Inorganic P	All		-0.42**	-0.41**	-0.19	0.22	-0.07	0.22	0.24	-0.38*	-0.27	-0.05	0.08	0.28	-0.04	-0.24
(mg g^{-1})	BC		-0.42*	-0.24	0.37*	0.04	-0.25	-0.02	0.40	-0.42*	0.09	-0.21	-0.23	0.22	0.26	-0.11
Emergence	All			0.70**	-0.16	0.02	0.28	-0.09	-0.33*	0.37*	-0.08	-0.17	0.10	0.06	-0.07	-0.09
(%)	BC			0.74**	-0.54**	-0.10	0.19	0.23	-0.21	0.22	0.15	-0.04	-0.06	0.03	0.11	0.05
Yield	All				0.39*	0.34*	0.65**	-0.37*	-0.61**	0.56**	-0.37*	-0.49**	0.34*	0.34*	-0.39*	-0.39*
(kg ha^{-1})	BC				-0.23	0.09	0.31	0.40*	-0.39*	0.36*	0.15	-0.14	-0.27	0.27	0.13	-0.02
Maturity	All					0.40*	0.51**	-0.43**	-0.28	0.21	-0.33*	-0.41**	0.31*	0.28	-0.26	-0.35*
(days†)	BC					0.28	0.12	-0.02	0.04	-0.10	-0.03	-0.01	-0.10	0.09	0.12	-0.02
Lodging	All						0.81**	-0.33*	-0.39*	0.32*	-0.83**	-0.73**	0.53**	0.77**	-0.20	-0.82**
(score‡)	BC						0.53**	0.30	-0.41*	0.40*	-0.09	0.26	-0.19	0.14	0.17	0.14
Height	All							-0.55**	-0.55**	0.48**	-0.84**	-0.82**	0.66**	0.75**	-0.44**	-0.85**
(cm)	BC							0.32	-0.60**	0.58**	0.17	0.41*	-0.16	0.12	-0.07	0.39*
Seed size	All								0.26	-0.16	0.53**	0.57**	-0.51**	-0.43**	0.37*	0.54**
(mg sd^{-1})	BC								-0.10	0.22	-0.17	-0.14	-0.04	0.06	0.06	-0.19
Protein	All									-0.95**	0.22	0.14	-0.19	-0.22	0.45**	0.21
(g kg^{-1})	BC									-0.96**	-0.29	-0.54**	0.21	-0.16	0.18	-0.54**
Oil	All										-0.14	-0.08	0.13	0.13	-0.36*	-0.13
(g kg^{-1})	BC										0.21	0.57**	-0.14	0.10	-0.20	0.52**

Table H1. Continued.

Trait	Lines§	Inorganic P	Emergence	Yield	Maturity	Lodging	Height	Seed size	Protein	Oil	Palmitate	Stearate	Oleate	Linoleate	Linolenate	Saturates
Palmitate	All											0.93**	-0.73**	-0.89**	0.32*	1.00**
(g kg^{-1})	BC											0.28	-0.04	-0.04	-0.26	0.72**
Stearate	All												-0.67**	-0.86**	0.25	0.95**
(g kg^{-1})	BC												0.16	-0.27	-0.33*	0.87**
Oleate	All													0.35**	-0.69**	-0.73**
(g kg^{-1})	BC													-0.98**	-0.66**	0.10
Linoleate	All														-0.05	-0.90**
(g kg^{-1})	BC														0.59**	-0.22
Linolenate	All															0.32*
(g kg^{-1})	BC															-0.37*

* coefficients were significant at the 0.05 probability level.
** coefficients were significant at the 0.01 probability level.
† = days after 31 August.
‡ = score 1 (all plants erect) to 5 (all plants prostrate).
§= All = backcross lines, parents, and check, BC = backcross lines only.

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