Inositol Metabolism in Developing Seed of Low and Normal Phytic Acid Soybean Lines

Daniel W. Israel, Earl Taliercio, Prachuab Kwanyuen, Joseph W. Burton,* and Lisa Dean

ABSTRACT
While inositol has key roles in phytic acid and raffinosaccharide synthesis, its concentration in developing seed of low phytic acid soybean [Glycine max (L.) Merr.] lines derived from CX1834 (Wilcox et al., 2000) has not been compared to that of normal lines. Concentrations of metabolites in the phytic acid and raffinosaccharide biosynthesis have been measured in mature seed of CX1834-derived lines but not throughout seed development. Our objective was to compare concentrations of inositol and metabolites associated with phytic acid and raffinosaccharide synthesis in developing seed of CX1834-derived and normal lines. Plants were cultured with complete nutrient solutions in growth chambers with 650 to 700 μmol m⁻² s⁻¹ of photosynthetically active radiation and a 26/22°C day/night temperature. Seed inositol concentrations were high (60 to 90 mmol kg⁻¹ seed dry wt.) at 20 d after flowering (DAF) and decreased 95% by maturity in both normal and low phytic acid lines. In two of three experiments, low phytic acid lines had significantly (p ≤ 0.05) greater seed inositol concentrations than normal lines at the first two sampling dates, but differences at maturity were not significant. Seeds of low phytic acid and normal lines had statistically similar concentrations (p > 0.05) of partially phosphorylated inositol intermediate (inositol triphosphate [IP3]), stachyose, raffinose, and phytase activity throughout development. These results corroborate previous studies that ruled out defects in genes coding myo-inositol-1-P synthase, inositol kinases, and phytase as the basis for the low seed phytic acid trait in CX1834-derived lines.

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When inositol accumulation was impaired by a mutation in the \textit{myo}-inositol–1-P synthase (MIPS) gene, a 50% reduction in phytic acid concentration and an 80% reduction in raffinosaccharide concentration in mature soybean seed were measured (Hitz et al., 2002). Thus, although inositol is not incorporated into raffinosaccharide molecules, it has a critical role in their synthesis. Bilyeu et al. (2008) reported that mature seed raffinosaccharide concentration in a low phytic acid line (CX1834), which has been used as a low phytic acid seed parent in breeding programs, was similar to that of normal phytic acid lines. This result indicates that the low phytic acid seed trait in CX1834 or in lines derived from it is not the result of a mutation in the MIPS gene. Although not measured in maturity group VII lines derived from CX1834, raffinosaccharide concentrations similar to seed of normal phytic acid lines would be expected.

Considerable research has been conducted on the impacts of genetic alterations on phytic acid and raffinosaccharide biosynthesis in soybean seed (Hitz et al., 2002; Israel et al., 2007; Oltmans et al., 2004; Wilcox et al., 2000). Inositol concentrations in mature seed of normal and low phytic acid lines of soybean and maize (\textit{Zea mays} L.) have been reported (Hitz et al., 2002; Karner et al., 2004; Shi et al., 2003, 2005), but changes in seed inositol concentrations have not been monitored throughout reproductive development. Karner et al. (2004) reported that in barley (\textit{Hordeum vulgare} L.) mature seed of low phytic acid lines (lpa1-1 and lpa2-2) had phytic acid concentrations 10 to 12 mmol kg\(^{-1}\) seed dry wt. less than normal phytic acid lines. While inositol concentrations in mature seed increased significantly in these low phytic acid lines compared to normal lines, the magnitude of the increase was only 1.5 mmol kg\(^{-1}\) dry wt. (Karner et al., 2004). Thus, measuring inositol concentrations in mature seed does not reflect the level of inositol metabolism in low and normal phytic acid lines. Seed inositol concentrations have not been reported for CX1834- or CX1834-derived lines at any stage of development. Because seed raffinosaccharide concentrations were similar in CX1834- or CX1834-derived lines and normal phytic acid lines (Bilyeu et al., 2008), similar seed inositol concentrations would be expected in these lines (Bilyeu et al., 2008).

Phytases degrade phytic acid during seed germination to provide phosphorus to support growth of the seedling. Phytic acid concentrations in mature seed decreased 90% on expression of a bacterial phytase in soybean 'Jack' (Bilyeu et al., 2008). Phytase activity in mature seed of the low phytic acid line CX1834 (Wilcox et al., 2000) was similar to that of normal phytic acid lines (Bilyeu et al., 2008). Thus, current information suggests that altered phytase activity does not account for the low phytic acid trait in CX1834 or lines derived from it. However, seed phytase activity has not been measured throughout seed development of these low seed phytic acid lines.

Our objective was to characterize changes in seed inositol concentrations throughout reproductive development of low phytic acid soybean lines derived from CX1834 and normal phytic acid lines in relation to changes in metabolites associated with phytic acid and raffinosaccharide synthesis. This information could provide insight about the magnitude of inositol utilization in phytic acid synthesis in developing soybean seed and how decreased inositol utilization in phytic acid synthesis in low phytic acid lines impacts the concentration of raffinosaccharides in the seed.

**MATERIALS AND METHODS**

**Experimental Treatments**

Three experiments were used to characterize seed metabolite concentrations throughout reproductive development. Low phytic acid genes in lines used in these experiments were derived from soybean genotype CX1834. Mutant M153 derived from mutagenesis with ethyl methane-sulfonate was the source of the low phytic acid trait in CX1834 (Wilcox et al., 2000). In Exp. 1, two soybean genotypes, Haskell-RR (normal phytic acid) (Boerma et al., 1994) and a low phytic acid genotype in a Haskell-RR genetic background (a BC\(_4\)-derived line homozygous for low phytic acid alleles at loci on linkage groups [LGs] L and N), were grown in controlled environment chambers. Two P treatments were imposed, 0.05 mmol L\(^{-1}\) (P deficient) and 0.5 mmol L\(^{-1}\) (P sufficient). We had shown previously (Israel et al. 2007) that increasing external P supply from deficient to sufficient levels increased seed phytic acid concentrations in normal lines and seed inorganic P concentrations in low phytic acid lines. However, the impact of external P supply on seed inositol and raffinosaccharide concentrations was not evaluated in our previous studies. Seed samples for metabolite measurements were taken at 20, 29, and 63 d after flowering (DAF), which occurred 35 d after planting. The P treatment solutions were prepared in phytotron nutrient solution (Thomas and Downs, 1991) modified to contain 15 mmol L\(^{-1}\) N with 25% as NH\(_4\)–N and 75% as NO\(_3\)–N. Only the sufficient level of P (0.5 mmol L\(^{-1}\)) was used in Exp. 2 and 3 because increasing external P concentration in Exp. 1 increased seed levels of phytic acid in the normal genotype and phosphate in the low phytic acid genotype (Fig. 1) as expected from previous work (Israel et al., 2007). In Exp. 2, the same low and normal phytic acid seed lines were used and seed samples were taken on four dates during reproductive development (19, 26, 33, and 59 DAF). An additional line with normal phytic acid seed (Prichard-RR) and a line with low seed phytic acid seed in a Prichard-RR genetic background (GO3PHY-443 RR, a BC\(_4\)-derived line homozygous for low phytic acid alleles at loci on LGs L and N) were included in Exp. 3. These lines flowered at 35 DAF under the environmental conditions in the growth chambers. Seed samples were taken at 19, 30, and 58 DAF.

**Plant Culture Conditions**

Three seeds were planted 2.5-cm deep in 9-L pots filled with moist potting mix (2:1 gravel:peat-lite v:v). One week after emergence, plants were thinned to one healthy seedling per pot. A 9-h photoperiod with light intensities that ranged from

\[ \text{...} \]
were used for collection of seed at each sampling date so that changes in reproductive load could not impact seed metabolite levels at successive sampling dates. Samples were immediately placed on ice and within 1 h transferred to a −80°C freezer for storage until extractions and analyses could be performed. In Exp. 2 and 3, seeds were frozen in liquid nitrogen immediately after removal from pods and transferred to a −80°C freezer for storage. Samples were freeze-dried and ground to pass a 0.5-mm screen. For determination of phytic acid and partially phosphorylated intermediates of inositol (inositol triphosphates [IP3], inositol tetraphosphates [IP4], and inositol pentaphosphates [IP5]), 0.5-g dry wt. samples were extracted with 10 mL of 0.5 mol L−1 HCl, centrifuged, and passed through a 0.45-μm filter. Phytic acid and inositol phosphates in extracts were separated on a binary high performance liquid chromatography (HPLC) system and detected by absorbance at 550 nm after post column reaction with Wade’s color reagent (Kwanyuen and Burton, 2005; Wade and Morgan, 1955). Inorganic P was determined by a modification of the method of Larson et al. (2000) as described by Israel et al. (2007).

Soluble sugars (inositol, glucose, fructose, sucrose, raffinose, and stachyose) in ground seed material (0.1 g) were extracted in 15 mL of extraction buffer with stirring for 30 min. The extraction buffer consisted of methanol:chloroform:water in a 1:1:2.5 ratio. After extraction, aliquots were passed through a 0.45-μm filter and stored at −80°C until analyses were conducted. Glucose and fructose were determined using the glucose oxidase and peroxidase assay kit (Cayman Chemical Co., Ann Arbor, MI), and sucrose and raffinose were determined using a refractometer (Bausch & Lomb, Rochester, NY). Inositol was determined with a modification of the method of Hümmer et al. (2005). Stachyose was determined by HPLC (see “Phytate and partially phosphorylated intermediates of inositol” section).

Figure 1. Impact of the low seed phytic acid trait and external P supply on concentrations of metabolites associated with phytic acid synthesis in developing soybean seed (Exp. 1). Error bars about data points are standard deviations of the mean.
(12:5:3 v:v:v) and 53.3 mg L\(^{-1}\) lactose and 26.7 mg L\(^{-1}\) cellobiose as internal standards. The samples were centrifuged at 4100 \(\times g\) and the supernatant solution containing soluble sugars was poured into a new tube. The volume of extract was reduced by placing uncapped tubes in a fume hood overnight before drying in a vacuum oven at 37°C. The sugars were dissolved in 2 mL of HPLC-grade water and a 50-μL aliquot was diluted to 2 mL with water. Dilute samples were passed through a Dionex On Guard filter (Dionex Corporation, Sunnyvale, CA) and 1 mL was collected in an HPLC auto sampler vial. Soluble sugars were separated by HPLC and quantified by pulsed amperometric detection (Pattee et al., 2000).

Phytase was isolated from seed taken in Exp. 3 and the activity determined as described by Chiera et al. (2004) with slight modifications. Samples (0.15 g) were extracted with 1.5 mL of pH 4.5 buffer containing 50 mmol L\(^{-1}\) sodium acetate, 20 mmol L\(^{-1}\) CaCl\(_2\), 1 mmol L\(^{-1}\) dithiothreitol, and 1 mmol L\(^{-1}\) phenylmethylsulforyl fluoride in microfuge tubes that were rotated for 30 min at room temperature. Samples were centrifuged at 18,000 \(\times g\) for 20 min. Each supernatant was dialyzed in 1.6 L of 50 mmol L\(^{-1}\) sodium acetate buffer, pH 5.5, for 24 h at 4°C with at least one change of dialysis buffer. The dialysis membrane had a molecular weight cutoff of 5000 Da. Samples were then centrifuged at 18,000 \(\times g\) for 20 min. Total protein concentration of the supernatant was determined as described by Bradford (1976). Phytase activity was measured by incubating 100 μL of protein extract with 100 μL of 1 mmol L\(^{-1}\) sodium phytate and 300 μL of 50 mmol L\(^{-1}\) sodium acetate buffer, pH 5.5, at 55°C for 10 min. The phytase reaction was stopped by adding 500 μL of 0.4 mol L\(^{-1}\) HCl, and phosphorus liberated by the enzyme was reacted with 1 mL of freshly prepared Chen’s reagent (Chen et al., 1956) which contained one part 6 N H\(_2\)SO\(_4\), one part 20 mmol L\(^{-1}\) ammonium molybdate, one part 570 mmol L\(^{-1}\) ascorbic acid, and two parts water. Absorbance was then measured at 802 nm after color was allowed to develop for 10 min. Phosphorus concentration was determined from a standard curve using known concentration of monobasic potassium phosphate.

In all experiments, treatment combinations were replicated three times and arranged in a randomized block design. The SAS GLM procedure (SAS Institute, 1999) was used for statistical analyses. Genotype, external P level, and sampling date were considered fixed in the tests. Residual mean square was used for testing main effects and interactions.

### RESULTS

Phosphorus deficiency significantly decreased seed concentrations of phytic acid in both the normal and low phytic acid genotypes (Table 1; Fig. 1). While genotype (phytic acid trait) and P supply had significant effects on seed inositol concentration, absolute differences were small and large decreases in concentration occurred during the course of seed development (Table 1; Fig. 1). Small genotypic differences were not associated with the low phytic acid trait (Fig. 1). Other partially phosphorylated inositol intermediates (IP4 and IP5) were not detected.

Concentrations of sucrose, stachyose, and raffinose were measured during seed development (Fig. 2). In all genotype by P treatment combinations, sucrose concentrations decreased to about the same extent between 20

### Table 1. Mean squares for ANOVA of phosphorus, genotypic, and time (days after flowering [DAF]) effects on soybean seed metabolite concentrations throughout reproductive development of genotypes that produce seed with normal and low phytic acid concentrations.

<table>
<thead>
<tr>
<th>Treatment effects</th>
<th>Phytic acid</th>
<th>Phosphate</th>
<th>Inositol P-3</th>
<th>Inositol</th>
<th>Sucrose</th>
<th>Stachyose + Raffinose</th>
<th>Phytase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>156**</td>
<td>5335**</td>
<td>0.366*</td>
<td>276*</td>
<td>62</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>P level (P)</td>
<td>297**</td>
<td>8084**</td>
<td>1.155**</td>
<td>379**</td>
<td>6628**</td>
<td>31</td>
<td>–</td>
</tr>
<tr>
<td>DAF†</td>
<td>430*</td>
<td>24*</td>
<td>2.808**</td>
<td>12421**</td>
<td>24315**</td>
<td>9249**</td>
<td>–</td>
</tr>
<tr>
<td>G × P</td>
<td>85**</td>
<td>4640**</td>
<td>0.692*</td>
<td>1</td>
<td>170</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>G × DAF</td>
<td>43**</td>
<td>1073**</td>
<td>0.292*</td>
<td>74</td>
<td>106</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td>P × DAF</td>
<td>73**</td>
<td>160**</td>
<td>0.078</td>
<td>141</td>
<td>661</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>G × DAF × P</td>
<td>32**</td>
<td>933**</td>
<td>0.022</td>
<td>76</td>
<td>43</td>
<td>21</td>
<td>–</td>
</tr>
<tr>
<td>Exp. 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>229*</td>
<td>5263**</td>
<td>–</td>
<td>37</td>
<td>1</td>
<td>7</td>
<td>–</td>
</tr>
<tr>
<td>DAF</td>
<td>335**</td>
<td>4</td>
<td>–</td>
<td>5727**</td>
<td>7478**</td>
<td>5742**</td>
<td>–</td>
</tr>
<tr>
<td>G × DAF</td>
<td>23**</td>
<td>548**</td>
<td>–</td>
<td>10</td>
<td>127</td>
<td>10</td>
<td>–</td>
</tr>
<tr>
<td>Exp. 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>237**</td>
<td>4537**</td>
<td>–</td>
<td>62*</td>
<td>79</td>
<td>92**</td>
<td>0.34*</td>
</tr>
<tr>
<td>DAF</td>
<td>754**</td>
<td>6</td>
<td>–</td>
<td>17967**</td>
<td>17068**</td>
<td>–¹</td>
<td>38.35**</td>
</tr>
<tr>
<td>G × DAF</td>
<td>43**</td>
<td>1012**</td>
<td>–</td>
<td>53*</td>
<td>364**</td>
<td>–</td>
<td>0.54**</td>
</tr>
</tbody>
</table>

*Significant at 0.05 probability level.

**Significant at 0.01 probability level.

¹Only 58 DAF sampling was used in statistical analysis.
DAF and seed maturity (Fig. 2). Insufficient P supply significantly reduced the concentration of sucrose at the two early sampling times but did not significantly affect the seed raffinosaccharide concentration (Table 1, Fig. 2). The effects of genotype on sucrose and raffinosaccharide concentrations in seed were not significant (Table 1; Fig. 2). Raffinosaccharide (stachyose plus raffinose) concentrations increased to a similar extent in all treatments between 30 DAF and seed maturity (Fig. 2). Although sucrose concentration was significantly lower in the P limited treatment than in the adequate P treatment (Table 1) the difference was relatively small at maturity.

In Exp. 2 and 3 with low and normal phytic acid lines supplied nutrient solution with a sufficient P level (0.5 mmol L⁻¹), changes in sucrose and raffinosaccharide concentrations in developing seed (Fig. 3 and 4) were similar to those observed for the sufficient P treatment in Exp. 1 (Fig. 1). One exception was a significant genotypic effect on seed raffinosaccharide concentrations in Exp. 3 (Table 1; Fig. 4). Although seed raffinosaccharide concentrations at maturity varied by 13.5 mmol kg⁻¹ dry wt. among genotypes (Fig. 4) and were significantly different (Table 1), this variation was not associated with the low phytic acid trait as means for normal and low phytic acid lines were almost identical (54.8 vs. 55.5 mmol kg⁻¹ dry wt.).

A small but significant genotypic effect on seed phytase activity (Tables 1 and 2) was observed. However, the genotypic differences were not associated with the low phytic acid trait (Table 2). Phytase activity for all lines declined more than 50% between 20 DAF and maturity (Table 2).

**DISCUSSION**

Previous studies compared inositol concentrations in mature seed of normal and low phytic acid lines of soybean and maize (Hitz et al., 2002; Shi et al., 2003, 2005). A mutation in the MIPS gene decreased inositol concentration in mature soybean seed as well as phytic acid and raffinosaccharide concentrations (Hitz et al., 2002). Shi et al. (2005) demonstrated that a mutation in the genes of maize encoding an inositol kinase that catalyzes phosphorylation of inositol to generate multiple inositol monophosphates, enantiomers Ins(1/3)P and Ins(4/6)P, and possibly Ins(5)P resulted in a decrease in phytic acid and an increase in inositol concentrations in mature maize seed. Increased inositol concentrations were measured in mature maize seed with a mutation in the gene that encodes inositol monophosphate kinase (Shi et al., 2003).

In the present study, inositol concentrations in soybean seed were in the 60 to 90 mmol kg⁻¹ seed dry wt. range for both low and normal phytic acid lines at beginning seed fill (20 DAF) and decreased to similar low concentrations at maturity (Table 1; Fig. 1, 3, and 4). Seed raffinosaccharide concentrations were not significantly different in normal and low phytic acid lines (Table 1; Fig. 2, 3, and 4). Seed phytic acid concentrations were 10 to 13 mmol kg⁻¹ seed dry wt. less in low phytic acid lines than in normal lines (Fig. 1, 3, and 4). The greatest difference in inositol concentrations in mature seed of low and normal genotypes was 3 mmol kg⁻¹ seed dry wt. (Fig. 1, 3, and 4). These results indicate that neither inositol synthesis nor utilization is impaired in the low phytic acid lines. Apparently, inositol utilization in cellular processes other than phytic acid and raffinosaccharide synthesis is greater in low phytic acid lines than in normal lines.

Relatively small differences in seed inositol concentrations at 20 DAF (Fig. 1, 3, and 4), similar decreases in inositol concentration during seed maturation (Fig. 1, 3, and 4), and similar seed raffinosaccharide concentrations (Fig. 2, 3, and 4) were observed for low and normal phytic acid lines. These observations indicate that a mutation(s) in the MIPS gene would not account for the low phytic acid trait in lines derived from CX1834 (Wilcox et al., 2000).

**Figure 2.** Influence of external P supply and low seed phytic acid trait on concentrations of sucrose and raffinosaccharides in seed throughout reproductive development (Exp. 1). Since the genotypic effect on sucrose concentration was not significant at the 0.05 probability level data were averaged across genotypes for each P treatment and plotted as a function of sampling date. Error bars about data points are standard deviations of the mean.
The partially phosphorylated intermediate, IP₃, was present at low concentrations in seed at 20 DAF and decreased gradually during seed maturation in both low and normal phytic acid lines (Fig. 1). The IP₄ and IP₅ intermediates were barely detectable in seed of low and normal phytic acid lines (data not shown). Wilcox et al. (2000) reported that phosphorylated inositol intermediates were not elevated in mature seed of the low seed phytic acid mutant, M153, from which CX1834 was derived. These observations support the interpretation that mutations in genes encoding kinases that phosphorylate inositol do not account for the low phytic acid trait in lines derived from CX1834.

Bilyeu et al. (2008) demonstrated that seed phytic acid concentrations at maturity could be decreased by more than 95% by expressing a bacterial phytase in soybean seed. These authors also reported that phytase activities in mature seed of the low phytic acid line, CX1834, and a normal phytic acid line were not significantly different. In our present study, similar phytase activities in normal and low phytic acid lines throughout seed development (Table 2) indicate that enhanced expression of phytase activity does not account for the low phytic acid phenotype CX1834-derived lines.

Oltmans et al. (2004) demonstrated that the low seed phytic acid trait in soybean lines derived from the CX1834 line is conditioned by two recessive genes with duplicate dominant epistasis. Walker et al. (2006) used genetic mapping to show that these genes are on LGs L and N. Genotypes homozygous recessive for only one of the two genes (LGL and LGN) had increases in seed phytic acid concentration in response to increasing P supply equal to that of the normal phytic acid parental line (Israel et al., 2007). This observation is consistent with control of the low phytic acid trait by two recessive genes in CX1834-derived lines.

Recently, silencing expression of a gene coding for multidrug resistant-associated protein (MRP) adenosine triphosphate (ATP)-binding cassette (ABC) transporter caused a low seed phytic acid phenotype in maize (Shi et al., 2007). Saghai Maroof et al. (2009) used a comparative sequence analysis approach to identify genes encoding two MRPs on...
LGs L and N, which when mutated may be responsible for the low phytic acid trait. They also demonstrated that the gene associated with LG N has a point nucleotide mutation that leads to a substitution of T for an A that changes an arginine codon to a stop codon. Gillman et al. (2009) have identified a mis-sense mutation in a conserved amino acid in the CX1834 allele of LG L/chr 19 lpa1 homolog. The specific function of the MRP-ABC transporter genes in phytic acid metabolism is not known, but a possible role in movement of phytic acid, inorganic P, or an inositol phosphate intermediate between subcellular compartments in developing seed has been suggested (Raboy, 2007; Gillman et al., 2009).

Concentrations of inositol, inositol phosphate intermediates, raffinosaccharides, and phytase activity were similar throughout seed development of normal and low phytic acid lines (Table 2 and Fig. 1, 3, and 4). These results in conjunction with those of Bilyeu et al. (2008) and Wilcox et al. (2000) are consistent with genetic studies that implicate mutations in the MRP-ABC transporter genes as being responsible for the low seed phytic acid trait in soybean lines derived from CX1834 (Saghai Maroof et al., 2009; Gillman et al., 2009).

Table 2. Phytase activity (mean ± SD, n = 3) in seed of low and normal phytic acid soybean lines at different stages of reproductive development.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phytic acid phenotype</th>
<th>Days after flowering</th>
<th>Phytase activity, ng P min⁻¹ mg⁻¹ protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prichard RR</td>
<td>Normal</td>
<td>19, 30, 58</td>
<td>5.8 ± 0.2, 2.5 ± 0.2, 1.5 ± 0.3</td>
</tr>
<tr>
<td>GO3PHY-443 RR, lpa†</td>
<td>Low</td>
<td>19, 30, 58</td>
<td>4.9 ± 0.4, 2.5 ± 0.3, 2.7 ± 0.5</td>
</tr>
<tr>
<td>Haskell RR</td>
<td>Normal</td>
<td>19, 30, 58</td>
<td>5.7 ± 0.2, 3.0 ± 0.3, 2.3 ± 0.2</td>
</tr>
<tr>
<td>Haskell RR, lpa</td>
<td>Low</td>
<td>19, 30, 58</td>
<td>5.4 ± 0.1, 2.6 ± 0.5, 2.7 ± 0.5</td>
</tr>
</tbody>
</table>

†lpa, low phytic acid trait.

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References


