Effects of Four Independent Low-Phytate Mutations in Barley (Hordeum vulgare L.) on Seed Phosphorus Characteristics and Malting Quality

Phil Bregitzer1,2 and Victor Raboy1

ABSTRACT

Conversion of the seed phosphorus storage compound phytic acid, which is poorly digested by nonruminants, to available forms of phosphorus will have nutritional and environmental benefits. Low-phytate (LP) barley (Hordeum vulgare L.) cultivars are in development and their commercialization will be facilitated by understanding their phosphorus profiles and malting quality. To study these issues, LP and normal types derived from mutagenized populations of barley cultivar Harrington (sets of sib lines homozygous for the wild-type [WT] allele, or for one of four low-phytic acid mutations, ipa1-1, ipa2-1, ipa3-1, or M955), were developed through backcrosses to Harrington. Grain was produced in irrigated and rain-fed environments. WT phosphorus profiles were similar to those of Harrington, suggesting that the major variable was the presence or absence of mutant alleles. All mutations conferred increased inorganic phosphorus. Total P was reduced for ipa1-1. Phosphorus profiles were relatively stable across environments, which will facilitate the inclusion of LP barley in animal rations. Utilization of LP cultivars for malting may be difficult, as the LP trait was associated with substantial reductions in diastatic power. All mutations, except for ipa2-1, affected wort β-glucan levels, which could not be attributed to altered grain β-glucan levels.

To improve the mineral nutrition of monogastric animals and lessen the environmental impact associated with their production, low-phytate (LP) cultivars of several crops are being under development. Monogastric animals lack phytase, thus causing phytate P to be nutritionally unavailable and ultimately excreted as phosphate in feces (Leytem et al 2004) where it can serve as a significant source of surface and ground water pollution (Sharpley et al 2003). Furthermore, phytate P is an effective chelator of divalent cations and can contribute to mineral deficiencies (Ravindran et al 1995).

Compared to wildtype (WT) cultivars, in which the majority of seed phosphorus is stored as phytic acid (phytate; myo-inositol 1,2,3,4,5,6-hexakisphosphate) (Rabay 1997; Lott et al 2000), LP cultivars produce grain that has a significant increase in the ratio of available phosphorus to phytate P, generally without significant alterations in seed total P. The LP phenotypes are derived from various mutations that have been isolated in several species, including barley, rice, wheat, maize, and soybean (Rasmusen et al 1998; Raboy et al 2000, 2001; Wilcox et al 2000; Hitz et al 2002; Guttieri et al 2004). Feeding trials conducted with swine, fish, and poultry have associated the LP trait with improved phosphorus and mineral availability, growth rates, and reductions in fecal phosphorus (Ertl et al 1998; Spencer et al 2000; Li et al 2001a,b; Yeum et al 2002; Jang et al 2003; Overturf et al 2003; Thacker et al 2003; Leytem et al 2004).

Widespread production and adoption of LP cereals and legumes for feeding monogastric animals will be facilitated by thorough study of their agronomic and biochemical characteristics. This information is critical to understanding the economic opportunities and challenges offered by LP crops. Relevant issues to address include determinations of productivity and grain phosphorus profiles under different production environments and the effect of the LP mutations on other aspects of quality.

To better understand the characteristics of LP barley mutations, backcross-derived WT and LP sib sets were derived as a means of separating the loci conditioning the LP trait from other genomic changes that may have been present in the original mutants. WT and LP sib sets pairs derived from four lines containing independent LP mutations (phytate reduction range of 40–95% of WT) have been assessed for their agronomic performance (Bregitzer and Raboy 2006). These tests were conducted under two distinct environmental regimes: irrigated (low stress, high productivity) and rain-fed (high stress, low productivity). They showed mutation-dependent effects on agronomic performance. Moderate reductions in phytate had relatively small effects as compared with more extreme reductions. For this report, we have conducted additional analyses to assess the biochemical characteristics of these LP mutations. Specifically, we examined the phosphorus profiles of barley grain as influenced by each of the four mutations, and examined the stability of these profiles as influenced by irrigated (non-stressed) and rain-fed (stressed) production environments. In addition, grain samples from the irrigated environments were malted and characterized for major determinants of malting quality.

MATERIALS AND METHODS

Derivation of LP and WT Barley Lines

The four mutations studied were generated by sodium azide treatment of the barley cultivar Harrington, and their origins have been described in detail (Dorsch et al 2003). Total P (TP) levels were unchanged from WT, except for ipa1-1, which showed slightly reduced TP. Mutant lines containing the M955, ipa3-1, and ipa1-1 LP alleles had phytate reductions of ≈95, 65, and 50%, respectively, with a proportional increase in the amount of inorganic P (Pi). Mutant lines containing the ipa2-1 mutation had a phytate reduction of ≈40%, with proportional increases in a pool of non-phytate P that includes inositol phosphates with five or fewer phosphate esters (phytic acid has six phosphate esters per molecule) as well as Pi. Mapping studies have placed ipa1-1 in chromosome 2H, and ipa2-1 in chromosome 7H (Larson et al 1998). M955 and ipa3-1 have been mapped to chromosome 1H; it is not known whether they represent mutations of separate loci or allelic variants of the same locus (Roslinsky 2002).

The lines examined in this study were developed by backcrossing each mutant line to the wild-type (WT) parent, Harrington. The ipa1-1 lines were derived from BC3 populations, ipa2-1 and ipa3-1 from BC2 populations, and M955 from BC3 populations. Selections were based solely on the presence of the LP mutant allele in all backcross cycles except for the final one. For the final backcross cycle, F2,3 lines were grown in 2000 as 1.3-m headrows under irrigation at Aberdeen, ID. Heads were selected at random from rows that were visually similar to Harrington.

1National Small Grains Germplasm Research Facility, Agricultural Research Service, United States Department of Agriculture, 1691 S. 2700 W., Aberdeen, ID 83210. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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Heads homozygous for either the LP or the WT allele for each of the four mutations were identified based on Pi assays of five individual kernels as described earlier (Dorsch et al. 2003), and the remainder of the seed from each head was grown in 2001 as F<sub>1</sub>, in 1.3-m headrows at Aberdeen, ID. Individual headrows were selected based on visual similarity to Harrington and harvested in bulk. Their LP or WT status was verified as described above. For this study, six WT and six LP sibset lines (sibset pairs) were selected based on visual similarity to Harrington and harvested in 2001 as F<sub>1</sub>, Ipa<sub>3</sub>-1, lpa<sub>2</sub>-1, lpa<sub>1</sub>-1, or M<sub>955</sub>. Lines evaluated in 2002 and 2003 were BC<sub>1</sub>F<sub>3</sub> and BC<sub>2</sub>F<sub>3</sub> populations, respectively.

Test Sites
The 24 WT and 24 LP lines plus the Harrington WT parent were grown in small plots (2.5 x 1.5 m) at three trial location/years (subenvironments) that utilized irrigation (Aberdeen, ID, 2002 and 2003; Filer, ID, 2003) and at three trial location/years (subenvironments) that did not use irrigation (Teton, ID, 2002 and 2003; Soda Springs, ID, 2003). Prevaling agronomic practices for the growth of spring barley were utilized at each location as detailed in Bregitzer and Raboy (2006). These locations are diverse with respect to elevation, growing season, temperature, and water availability. In particular, the differences in water availability and, consequently, the level of moisture stress imposed by the different irrigation regimes were a significant factor affecting overall crop performance that revealed differences among the four mutations for agronomic performance and stress tolerance (Bregitzer and Raboy 2006).

Analytical Procedures for Determination of Grain and Malt Biochemical Characteristics
Grain samples (8–10 g) were ground to a uniform meal in a cyclone mill (Udy Corp., Fort Collins, CO) fitted with a 1.0-mm screen. Quantification of TP was done by wet-ashing (using sulfuric acid) 150 mg of meal and colorimetric assay of digest phosphorus (Chen et al. 1996). Quantification of inorganic P (Pi) was determined by extracting 50 mg of meal in 12.5% (w/v) trichloroacetic acid (TCA) 150 mg of meal and colorimetric assay of digest phosphorus (Chen et al. 1996). Grain β-glucan was determined according to Approved Method 32-23 (AACC International 2000) using the mixed-linkage β-glucan assay procedure (Megazyme International, Ireland), which involves sequential digestion with lichenase and β-glucosidase, and quantification of absorbance at 510 nm. Grain protein measurements were determined using an automated Dumas combustion procedure with a LECO FP-528 analyzer. Nitrogen values were converted to protein as N x 6.25.

Malt was prepared from grain samples (170 g, dry weight) and analyzed at the USDA-ARS Cereal Crops Research Unit, Madison, WI, following standard micromalting and analysis techniques. Barley samples (170 g, db) were steeped at 16°C for 32–48 hr to 45% moisture by alternating 4 hr of wet steep with 4 hr of air rest. The steeped samples were placed in a chamber for five days at 17°C and near 100% rh in cans that were rotated for 30 min every 30 min. The germinated grain (green malt) was kilned for 24 hr as follows: 0.5 hr from 25 to 49°C, 9.5 hr at 49°C, 0.5 hr from 49 to 54°C, 4.0 hr at 54°C, 0.5 hr from 54 to 60°C, 3.0 hr at 60°C, 0.5 hr from 60 to 68°C, 2.0 hr at 68°C, 0.5 hr from 68 to 85°C, and 3.0 hr at 85°C.

Malt extract was determined by the Malt-4 procedure (ASBC 1992), except that all weights and volumes specified for the method were halved. The specific gravity of the filtrate was measured with a density meter (Anton/Parr DMA 5000). The density data were used to calculate the amount of soluble material present in the filtrate, and thus the percentage that was extracted from the malt. Wort β-glucan levels were determined on a Skalar SAN plus analyzer by using the Wort-18 fluorescence flow injection analysis method with calcofluor as the fluorescent agent (ASBC 1992).

Total malt protein was determined as for grain protein (above). Soluble (wort) protein levels were determined on a Skalar SAN plus analyzer using the Wort-17 UV-spectrophotometric method (ASBC 1992). Diastatic power values were determined on a Skalar SAN plus analyzer by the automated ferricyanide procedure Malt-6A (ASBC 1992). α-Amylase activities were measured on a Skalar SAN plus analyzer by heating the extract to 73°C to inactivate any β-amylase present. The remaining (α-amylase) activity was measured as described for diastatic power values.

Experimental Design and Statistical Analysis
The experimental design at all locations was a randomized complete block with two replicates. Data were collected from all plots at all locations for Pi and TP. Malting quality data were obtained only from the irrigated locations: one replicate was analyzed for Aberdeen, 2002, and both replicates were analyzed for Aberdeen and Filer, 2003. Grain β-glucan determinations were made on

### TABLE I
Grain Phosphorus Characteristics of Wildtype (WT) and Low-Phytate (LP) Sibsets of Harrington Barley<sup>a,b</sup>

<table>
<thead>
<tr>
<th>Sibset Pair</th>
<th>Genotype</th>
<th>Irrigated</th>
<th>Rain-Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total P (mg/g)</td>
<td>Pi (mg/g)</td>
</tr>
<tr>
<td>lpa&lt;sub&gt;2&lt;/sub&gt;-1</td>
<td>WT</td>
<td>3.33</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>3.61a</td>
<td>0.89c</td>
</tr>
<tr>
<td></td>
<td>LP/WT</td>
<td>1.08</td>
<td>4.04c</td>
</tr>
<tr>
<td>lpa&lt;sub&gt;1&lt;/sub&gt;-1</td>
<td>WT</td>
<td>3.29</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>3.00b</td>
<td>1.06c</td>
</tr>
<tr>
<td></td>
<td>LP/WT</td>
<td>0.91</td>
<td>4.82&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>lpa&lt;sub&gt;3&lt;/sub&gt;-1</td>
<td>WT</td>
<td>3.46</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>3.49a</td>
<td>1.86b</td>
</tr>
<tr>
<td></td>
<td>1.01</td>
<td>8.08</td>
<td>1.05</td>
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<tr>
<td>M955</td>
<td>WT</td>
<td>3.47</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>3.54a</td>
<td>2.63a</td>
</tr>
<tr>
<td></td>
<td>LP/WT</td>
<td>1.02</td>
<td>9.74</td>
</tr>
<tr>
<td>Harrington</td>
<td>WT</td>
<td>3.31</td>
<td>0.20</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>3.39</td>
<td>0.84</td>
</tr>
</tbody>
</table>


<sup>b</sup> Bold print indicates significant differences for WT vs. LP values. LP sibsets followed by the same letter are not significantly different.

<sup>c</sup> Sibset × subenvironment (environment) interaction existed for differences among sibsets.
pooled samples from each replicate for grain produced at Aberdeen and Filer. All data were analyzed using statistical software (v. 8.0, SAS Institute, Cary, NC). Sibset (lpa1-1, lpa2-1, lpa3-1, or M955), genotype (WT or LP), and environment (irrigated or rainfed) and their interactions were considered fixed; other sources of variance were considered random. Comparisons of WT sibsets to Harrington were based on Dunnett's tests for comparisons with a common control; comparisons among LP sibsets were based on Tukey's multiple comparison procedure. Comparisons of LP vs. WT genotypes were made using single degree of freedom contrasts. All declarations of significance were based on \( P < 0.05 \).

**RESULTS**

**Seed Phosphorus Characteristics**

Lines (sibsets) and subenvironments (environments) were not significant sources of variability for TP and Pi, except as discussed below. Among the LP sibsets, the environment x sibset interaction was significant for Pi. Comparisons of WT vs. LP performance showed the environment x sibset pair x genotype interaction to be significant for TP and Pi. Accordingly, the data are presented by genotype within sibset for irrigated and rain-fed environments.

For both the irrigated and rain-fed environments, the WT sibsets were not significantly different than Harrington for TP or Pi, except as discussed below. TP and Pi were numerically higher in the rain-fed environments vs. irrigated environments (Table I). However, despite the lack of environment x subenvironment interactions (all rain-fed subenvironments had numerically higher TP and Pi than all irrigated subenvironments), this difference was not statistically significant. Thus, the WT sibsets had phosphorus profiles similar to that of the WT Harrington parent, and these profiles were similar under both environmental conditions. This indicates that the primary difference between the backcross-derived WT and LP sibsets was the presence or absence of the LP alleles. Therefore, the effects of each LP mutation could be assessed on the basis of comparisons of genotype (WT vs. LP) and on the basis of comparisons among the LP sibsets.

Comparisons of WT vs. LP genotypes within sibsets showed that the lpa3-1 and M955 mutations did not affect TP. However lpa2-1 caused a slight decrease and lpa2-1 caused a slight increase in TP (Table I). Comparisons among the LP sibsets also indicated a reduction in TP in both environments for lpa2-1 relative to the other LP sibsets, consistent with the results of Dorsch et al. (2003). For lpa2-1, there was a significant sibset x subenvironment(environment) interaction: at a single location (Tetonia in 2003), TP for lpa2-1 (4.26 mg/g) was greater than TP for lpa1-1 (3.27 mg/g) and TP for lpa3-1 (3.83 mg/g). The alterations by lpa1-1 and lpa2-1 were relatively stable across environments, with LP sibsets containing lpa1-1 showing 91 and 85% of WT values for TP, respectively, for irrigated and rain-fed environments. For lpa2-1, LP sibsets showed 118 and 110% of WT values for TP (Table I).

Substantial variability in Pi was noted in WT vs. LP genotype comparisons and among the LP sibsets. All four LP sibsets showed significant increases in Pi (Table I). As reported from studies of the original mutant lines, M955 had the greatest increase, lpa1-1 and lpa2-1 had the least increase, and lpa3-1 had an intermediate increase in Pi. Significant subenvironment x sibset(environment) interaction was noted among LP sibsets grown under rain-fed conditions. This could be traced to a significantly lower value for Pi in lpa1-1 (1.22 mg/g), relative to lpa2-1 (1.59 mg/g), at Soda Springs. However, the general relationship among the LP sibsets, in ascending order for the amount of Pi, was lpa2-1 = lpa1-1 < lpa3-1 < M955. As seen for TP, the WT vs. LP relationships for Pi were relatively stable across environments (Table I).

**Malting Quality**

Malting quality characteristics were not examined in detail for grain produced under the rain-fed environments. Grain produced under these environments was characterized by small kernels with high protein content (>17%), which prevents meaningful determinations of several important malting characteristics. Therefore, malting quality was determined only on grain produced in the three irrigated subenvironments. Measurements for selected characteristics are presented in Table II.

Harrington, the background cultivar in which the four mutations were originally induced and the recurrent parent used for the development of the populations studied for this report, is the North American standard two-rowed cultivar for malting quality, as defined by the American Malting Barley Association (AMBA). Comparison of AMBA specifications (http://www.ambainc.org/np/index.htm) to measurements of Harrington produced under irrigation (Table II) showed that it was of reasonably good quality. None of the WT sibsets differed significantly from Harrington for malt quality parameters analyzed for this report, with the exception of wort and grain α-glucan content.

**TABLE II**

Selected Malting and Grain Quality Characteristics of Wildtype (WT) and Low-Phytate (LP) Sibsets of Harrington Barley

<table>
<thead>
<tr>
<th>Sibset</th>
<th>Genotype</th>
<th>Plump Kernels* (%)</th>
<th>Kernel Weight (mg)</th>
<th>Malt Extract (%)</th>
<th>Barley Protein (%)</th>
<th>Soluble/Total Protein (%)</th>
<th>Diastatic Power (°ASBC)</th>
<th>α-Amylase (20° DU)</th>
<th>Wort β-Glucan (ppm)</th>
<th>Grain β-Glucan (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lpa1-1</td>
<td>WT</td>
<td>85</td>
<td>44</td>
<td>80.2</td>
<td>13.1</td>
<td>38</td>
<td>112</td>
<td>72</td>
<td>281</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>88</td>
<td>44a</td>
<td>80.1a</td>
<td>13.6a</td>
<td>39a</td>
<td>80a</td>
<td>70a</td>
<td>285a</td>
<td>–</td>
</tr>
<tr>
<td>lpa2-1</td>
<td>WT</td>
<td>82</td>
<td>45</td>
<td>80.4</td>
<td>13.5</td>
<td>39</td>
<td>118</td>
<td>71</td>
<td>230</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>83</td>
<td>43b</td>
<td>79.7a</td>
<td>13.3a</td>
<td>42a</td>
<td>98a</td>
<td>71a</td>
<td>101b</td>
<td>5.9</td>
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<tr>
<td>lpa3-1</td>
<td>WT</td>
<td>82</td>
<td>44</td>
<td>80.5</td>
<td>13.1</td>
<td>41</td>
<td>108</td>
<td>75</td>
<td>175</td>
<td>4.4</td>
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<tr>
<td></td>
<td>LP</td>
<td>85</td>
<td>43b</td>
<td>79.6a</td>
<td>13.2a</td>
<td>40a</td>
<td>88a</td>
<td>72a</td>
<td>275a</td>
<td>4.8</td>
</tr>
<tr>
<td>M955</td>
<td>WT</td>
<td>77</td>
<td>44</td>
<td>79.7</td>
<td>13.2</td>
<td>38</td>
<td>118</td>
<td>71</td>
<td>244</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>LP</td>
<td>89</td>
<td>44c</td>
<td>78.5a</td>
<td>13.9a</td>
<td>37a</td>
<td>84a</td>
<td>74a</td>
<td>125b</td>
<td>5.1</td>
</tr>
</tbody>
</table>


** Bold print indicates significant differences for WT vs. LP values. LP sibsets followed by the same letter are not significantly different.

% Percentage of kernels remaining on a 2.35- x 19.1-mm screen.

d Sibset x subenvironment(environment) interaction existed for differences among sibsets.

e Genotype x subenvironment interaction existed for differences among sibsets.
DISCUSSION

A major objective of this study was to examine the phenotypic stability, with respect to seed phosphorus profiles, of four LP mutations grown under conditions that are typical of a range of commercial production environments in the Intermountain West region of the United States. A secondary objective was to assess the effect of these mutations on malting quality. Previous agronomic analyses of these populations showed that the test locations provided diverse growing conditions that could be divided into two major types of environments: a low-stress, high-productivity irrigated environment and a high-stress, low-productivity environment (Bregitzer and Raboy 2006). Agronomic performance ranged from nearly equal to Harrington for Ipa1-I, Ipa2-I, and Ipa3-I under irrigation to markedly poorer performance under the stress of the rain-fed environment for Ipa2-I, Ipa3-I, and particularly so for M955.

Relatively few backcross cycles were used to develop the LP and WT sibsets, so individual WT and LP lines within a sibset pair were not truly near-isogenic lines. However, the representation of each genotype by six sibset lines allowed fair comparisons of WT vs. LP performance. Furthermore, for traits that showed changes (with the exception of grain β-glucan for Ipa3-I), the recovery of the recurrent parent performance in the WT sibsets indicated that the primary source of variability between WT and LP genotypes within a sibset was allelic variation at the locus conditioning the LP trait.

In general, total P and Pi profiles were relatively stable across divergent environments. Although there was a trend for increased TP and Pi in grains produced in rain-fed environments, the relative relationships between WT and LP genotypes within each sibset was remarkably stable. This has positive implications for the development of LP barley as an added-value commodity, as it will enable predictability in terms of utilizing LP barley as part of a feed ration to meet particular phosphorus nutrition requirements.

Most of the malting quality characteristics measured in this study were either unchanged or changed very slightly. Generally these changes were in the direction of reduced malt quality, with the exception of malt β-glucan for Ipa3-I, the recovery of the recurrent parent performance in the WT sibsets indicated that the primary source of variability between WT and LP genotypes within a sibset was allelic variation at the locus conditioning the LP trait.

Differences in the genetic constitution of LP genotypes compared with WT genotypes were noted among the LP sibsets, resulting in rankings for kernel weight of lpa2-1 > lpa1-1 = lpa3-1 > M955. Malt extract percentage reductions were not statistically significant, except for M955 at Filer, where the LP genotype had lower extract than the WT genotype (77.6 vs. 79.5%), and the LP sibset had lower extract than the other LP sibsets (79.2, 80.4, and 79.6 for lpa1-1, lpa2-1, and lpa3-1, respectively). Slight reductions in α-amylase activity were noted in comparisons of LP vs. WT genotypes for lpa3-1 and M955. A sibset × environment interaction was noted in comparisons among LP sibsets that could be traced to lower α-amylase for lpa1-1 at Aberdeen, 2002 (61 vs. 70, 68, and 67 for lpa2-1, lpa3-1, and M955, respectively) and for lpa2-1 at Filer (70 vs. 76, 75, and 78 for lpa1-1, lpa3-1, and M955, respectively).

Comparisons of LP vs. WT genotypes for diastatic power (DP) revealed significant reductions for all sibsets that were substantial in terms of their effect on malting quality (Table II). However, sibset × environment interactions were significant, and diastatic power was significantly reduced in all three subenvironments only for M955. For lpa1-1 and lpa2-1, significant reductions of diastatic power were noted in 2003 at both Aberdeen (82 vs. 106 for lpa1-1 and 76 vs. 103 for lpa2-1) and Filer (92 vs. 119 for lpa1-1 and 75 vs. 120 for lpa2-1). For lpa3-1, reductions were significant only at Filer in 2003 (89 vs. 114).

Measurements of wort β-glucan content showed that this characteristic was not necessarily recovered to Harrington levels in the WT sibsets. For lpa2-1 and M955, the WT sibsets had numerically higher values; however, significant differences from the control were noted only in a single subenvironment (Filer 2003). In comparisons of WT vs. LP genotypes, genotype × subenvironment interactions were noted. For lpa2-1, mean wort β-glucan did not differ but the WT sibsets had significant reductions at one subenvironment (Aberdeen 2002) and significant increases in another (Filer 2003). For lpa1-1, LP sibsets showed significantly lower values in two of the three subenvironments (Aberdeen and Filer 2003). The increases noted for LP lpa3-1 sibsets and the decreases noted for LPA M955 sibsets were significant in all subenvironments.

Differences in wort β-glucan content could derive from differences in grain β-glucan content or in the activities of β-glucanases during the malting process. In addition, variability in other modifications that take place during the malting process, such as the rate of water uptake, could contribute to variability in wort β-glucan content that is not directly related to variability in the activity of β-glucanases. For lpa1-1, lpa3-1, and M955, which showed significant differences in wort β-glucan content, measurements of grain β-glucan were conducted. Grain β-glucan contents for WT sibsets for lpa1-1 and M955 were not significantly different than Harrington. Comparisons of WT vs. LP genotypes showed that for lpa1-1, the LP genotype did not differ from WT. But for M955, LP genotype had significantly lower values. For lpa3-1, the WT genotype showed significant decreases in two of the three subenvironments compared with Harrington, and compared with the LP genotype.

These changes in grain β-glucan did not correspond to the changes noted for wort β-glucan. For lpa1-1, wort β-glucan was 44% and grain β-glucan was 104% of WT values; for lpa3-1, wort β-glucan was 157% and grain β-glucan was 108% of WT values; and for M955, wort β-glucan was 51% and grain β-glucan was 94% of WT values.
phytate P for WT, 32.5% phytate-P for lpaI-1). This level of aP, in combination with typical rations containing 15–20% soybean meal (0.18% aP), is sufficient to meet the phosphorus requirements (NRC 1998) of swine in the early finisher (50–80 kg; 0.19% aP required) and late finisher (80–20 kg; 0.15% aP required) stages. For this application, feeding barley with greater reductions in phytate P (such as M955) may not result in significant reductions in manure phosphorus content, a contention that is supported by the results of a study conducted by Leytem et al (2004). In this study, measurements of manure phosphorus contents from 50-kg swine that were fed essentially all-barley diets (WT, lpaI-1, lpa3-1, or M955) showed substantial reductions of total manure phosphorus for all LP barley diets relative to WT barley diets, but relatively small differences in manure phosphorus were detected among the three LP diets (Leytem et al 2004). This result is consistent with the interpretation that lpaI-1 barley supplied sufficient phosphorus for dietary needs and that the animals could not fully utilize the additional aP from lpa3-1 and M955. For this application, barley cultivars containing the lpaI-1 mutation may represent an ideal blend of agronomic performance and enhanced phosphorus availability.

CONCLUSIONS

Studies of four independent low-phytate (LP) mutations showed that lpaI-1 had reduced, and lpa3-2 increased, total P as compared with wild-type (WT). Inorganic P (Pi) showed substantial increases compared with WT values for all four mutations, and there was significant variability for Pi among the mutations. The increases in Pi are sufficiently large that they can contribute significantly to the phosphorus nutritional needs of nonruminant animals.

The phosphorus profiles were not significantly different in grain grown under divergent environmental conditions, although there was a tendency for both total P and Pi to be increased under rain-fed conditions. Thus, the expression of the LP trait appears to be relatively stable, a characteristic that will facilitate efficient formulation of rations that maximize the utilization of grain phosphorus and minimize manure phosphorus.

Measurements of malting quality showed either no changes or reduced quality; particularly important was a substantial decrease in diastatic power. Therefore, it may be difficult to develop a dual-use cultivar that combines both good malting quality and increased available phosphorus.

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LITERATURE CITED


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