

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

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ABSTRACT

Cereal Chem. 83(5):460–464

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, *lpa1-1*, *lpa2-1*, *lpa3-1*, 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 *lpa1-1*. 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 *lpa2-1*, affected wort  $\beta$ -glucan levels, which could not be attributed to altered grain  $\beta$ -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 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) (Raboy 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 (Rasmussen 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; Veum 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 sibsets 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 sibset 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 *lpa1-1*, which showed slightly reduced TP. Mutant lines containing the M955, *lpa3-1*, and *lpa1-1* LP alleles had phytate reductions of  $\approx$ 95, 65, and 50%, respectively, with a proportional increase in the amount of inorganic P (Pi). Mutant lines containing the *lpa2-1* mutation had a phytate reduction of  $\approx$ 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 *lpa1-1* in chromosome 2H, and *lpa2-1* in chromosome 7H (Larson et al 1998). M955 and *lpa3-1* 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 *lpa1-1* lines were derived from BC<sub>4</sub> populations, *lpa2-1* and *lpa3-1* from BC<sub>3</sub> populations, and M955 from BC<sub>2</sub> 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, F<sub>2,3</sub> 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.

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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>3,4</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 from each of the populations containing either *lpa1-1*, *lpa2-1*, *lpa3-1*, or M955. Lines evaluated in 2002 and 2003 were BC<sub>x</sub>F<sub>3,5</sub> and BC<sub>x</sub>F<sub>3,6</sub> populations, respectively.

### Test Sites

The 24 WT and 24 LP lines plus the Harrington WT parent were grown in small plots (2.5 × 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 (Tetonia, ID, 2002 and 2003; Soda Springs, ID, 2003). Prevailing 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 1956). Quantification of inorganic P (Pi) was determined by extracting 50 mg of meal in 12.5% (w/v) trichloroacetic acid in 0.2M MgCl<sub>2</sub>, followed by colorimetric assay of digest phosphorus (Chen et al 1956). 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 × 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 3.0 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 DMA5000). 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>

Sibset Pair	Genotype	Irrigated		Rain-Fed	
		Total P (mg/g)	Pi (mg/g)	Total P (mg/g)	Pi (mg/g)
<i>lpa 2-1</i>	WT	<b>3.33</b>	<b>0.22</b>	<b>4.02</b>	<b>0.26</b>
	LP	<b>3.61a</b>	<b>0.89c</b>	<b>4.43a<sup>c</sup></b>	<b>1.27c</b>
	LP/WT	1.08	4.04	1.10	4.88
<i>lpa 1-1</i>	WT	<b>3.29</b>	<b>0.22</b>	<b>3.99</b>	<b>0.26</b>
	LP	<b>3.00b</b>	<b>1.06c</b>	<b>3.39b</b>	<b>1.05c<sup>c</sup></b>
	LP/WT	0.91	4.82	0.85	4.03
<i>lpa 3-1</i>	WT	3.46	<b>0.23</b>	3.86	<b>0.26</b>
	LP	3.49a	<b>1.86b</b>	4.04a	<b>1.89b</b>
	LP/WT	1.01	8.08	1.05	7.26
M955	WT	3.47	<b>0.27</b>	4.04	<b>0.28</b>
	LP	3.54a	<b>2.63a</b>	4.24a	<b>3.04a</b>
	LP/WT	1.02	9.74	1.05	10.86
Harrington	WT	3.31	0.20	3.94	0.28
Mean		3.39	0.84	3.99	0.96

<sup>a</sup> Grown in Idaho under irrigation at Aberdeen (2002-2003) and Filer (2003) and under rain-fed conditions in Tetonia (2002-2003) and Soda Springs (2003).

<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 rain-fed) 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  $\times$  sibset interaction was significant for Pi. Comparisons of WT vs. LP performance showed the environment  $\times$  sibset pair  $\times$  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. TP and Pi were numerically higher in the rain-fed environments vs. irrigated environments (Table I). However, despite the lack of environment  $\times$  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 *lpa1-1* 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 *lpa1-1* relative to the

other LP sibsets, consistent with the results of Dorsch et al (2003). For *lpa2-1*, there was a significant sibset  $\times$  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 108 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  $\times$  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/ni/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  $\beta$ -glucan content.

TABLE II  
Selected Malting and Grain Quality Characteristics of Wildtype (WT) and Low-Phytate (LP)  
Sibsets of Harrington Barley<sup>a,b</sup>

Sibset	Genotype	Plump Kernels <sup>c</sup> (%)	Kernel Weight (mg)	Malt Extract (%)	Barley Protein (%)	Soluble/Total Protein (%)	Diastatic Power (°ASBC)	$\alpha$ -Amylase (20° DU)	Wort $\beta$ - Glucan (ppm)	Grain $\beta$ - Glucan (%)
<i>lpa 2-1</i>	WT	85	44	80.2	13.1	38	112	72	281	–
	LP	88	44a	80.1a	13.6a	39a	80a <sup>c</sup>	70a <sup>d</sup>	285a <sup>c</sup>	–
<i>lpa 1-1</i>	WT	82	45	80.4	13.5	39	118	71	230	5.7
	LP	83	43b	79.7a	13.3a	42a	98a <sup>c</sup>	71a <sup>d</sup>	101b <sup>d,e</sup>	5.9
<i>lpa 3-1</i>	WT	82	44	80.5	13.1	41	108	75	175	4.4
	LP	85	43b	79.6a	13.2a	40a	88a <sup>c</sup>	72a	275a	4.8
M955	WT	77	44	79.7	13.2	38	118	71	244	5.4
	LP	89	41c	78.5a <sup>d,e</sup>	13.9a	37a	84a	74a	125b	5.1
Harrington	WT	87	44	80.2	13.1	40	117	73	208	5.4

<sup>a</sup> Grown in Idaho under irrigation at Aberdeen (2002-3) and Filer (2003).

<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> Percentage of kernels remaining on a 2.38  $\times$  19.1-mm screen.

<sup>d</sup> Sibset  $\times$  subenvironment (environment) interaction existed for differences among sibsets.

<sup>e</sup> Genotype  $\times$  subenvironment interaction existed for WT vs. LP comparisons within sibsets.

Two important measures of malt quality (% barley protein and ratio of soluble to total protein) were not altered significantly for any of the LP sibsets. Several other characteristics (% plump kernels, kernel weight, malt extract %, and  $\alpha$ -amylase activity) showed minimal changes that were statistically significant in several instances. Percent plump kernels was reduced only in the LP vs. WT genotypes for M955. Kernel weight was slightly, but significantly, less in the LP vs. the WT genotypes for *lpa1-1* and M955 in two of the three subenvironments. Significant differences 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  $\alpha$ -amylase activity were noted in comparisons of LP vs. WT genotypes for *lpa3-1* and M955. A subset  $\times$  environment interaction was noted in comparisons among LP sibsets that could be traced to lower  $\alpha$ -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, subset  $\times$  subenvironment 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  $\beta$ -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  $\times$  subenvironment interactions were noted. For *lpa2-1*, mean wort  $\beta$ -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  $\beta$ -glucan content could derive from differences in grain  $\beta$ -glucan content or in the activities of  $\beta$ -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  $\beta$ -glucan content that is not directly related to variability in the activity of  $\beta$ -glucanases. For *lpa1-1*, *lpa3-1*, and M955, which showed significant differences in wort  $\beta$ -glucan content, measurements of grain  $\beta$ -glucan were conducted. Grain  $\beta$ -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  $\beta$ -glucan did not correspond to the changes noted for wort  $\beta$ -glucan. For *lpa1-1*, wort  $\beta$ -glucan was 44% and grain  $\beta$ -glucan was 104% of WT values; for *lpa3-1*, wort  $\beta$ -glucan was 157% and grain  $\beta$ -glucan was 108% of WT values; and for M955, wort  $\beta$ -glucan was 51% and grain  $\beta$ -glucan was 94% of WT values.

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 *lpa1-1*, *lpa2-1*, and *lpa3-1* under irrigation to markedly poorer performance under the stress of the rain-fed environment for *lpa2-1*, *lpa3-1*, 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  $\beta$ -glucan for *lpa3-1*), 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  $\beta$ -glucan for *lpa1-1* and M955. However, the reductions in diastatic power were substantial. High diastatic power is important for starch degradation during the brewing process, particularly for North American brewers that use significant quantities of adjunct grain (such as rice or corn). Although the observed reductions in diastatic power were not statistically significant in all environments except for M955, the trend was negative, and environmental sensitivity of a major determinant of malting quality is an undesirable characteristic in malting cultivars.

Although these mutations were studied in only a single genetic background, we have evaluated Pi alterations conditioned by all four of these mutations in a number of recombinant breeding lines of diverse parentage and, at least on a qualitative basis, the Pi relationships described by this study have been seen in all of these breeding lines (data not shown). However, additional studies are necessary to better understand the effect of genetic background of phosphorus profiles and malting quality.

These data provide empirical assessments of the available phosphorus in grain as influenced by four LP mutations. Knowledge of grain phosphorus characteristics, in combination with agronomic performance data (Bregitzer and Raboy 2006), enables breeders and animal producers a logical basis for assessing the utility of the available LP mutations. For instance, although the barleys with the *lpa1-1* mutation still contain significant amounts of phytate P, greater reductions may not be justified for some uses, particularly if such reductions are associated with reduced agronomic performance. For instance, assuming a reduction of phytate to  $\approx 50\%$  of WT levels in *lpa1-1* lines, LP grain produced under irrigated conditions showed a substantial increase in available P (aP), from  $\approx 1.2$  mg/g to 2.0 mg/g (based on aP = TP - phytate P; 65%

phytate P for WT, 32.5% phytate-P for *lpa1-1*). 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, *lpa1-1*, *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 *lpa1-1* 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 *lpa1-1* mutation may represent an ideal blend of agronomic performance and enhanced phosphorus availability.

## CONCLUSIONS

Studies of four independent low-phytate (LP) mutations showed that *lpa1-1* had reduced, and *lpa2-1* 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.

## ACKNOWLEDGMENTS

This research was supported by the Agricultural Research Service, USDA, project 5325-21430-006-00D, and project 5366-21000-021-00, and by the University of Idaho Research and Extension Centers at Aberdeen and Teton, ID. We wish to thank Clark Kaufman (Filer, ID) and Evan Hayes (American Falls, ID) for donating land and labor at the Filer and Soda Springs test sites.

## LITERATURE CITED

AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Method 32-23. The Association: St. Paul, MN.

ASBC. 1992. Malt-4, Wort-18, Wort-17, and Malt-6A. Methods of Analysis of the American Society of Brewing Chemists, 8th Ed. The Society: St. Paul, MN.

Bregitzer, P., and Raboy, V. 2006. Effects of four independent low-phytate mutations on barley agronomic performance. *Crop Sci.* 46:1318-1322.

Chen, P. S., Toribara, T. Y., and Warner, H. 1956. Microdetermination of phosphorus. *Anal. Chem.* 28:1756-1758.

Dorsch, J. A., Cook, A., Young, K. A., Anderson, J. M., Bauman, A. T., Volkmann, C. J., Murthy, P. P. N., and Raboy, V. 2003. Seed phosphorus and inositol phosphate phenotype of barley *low phytic acid* genotypes. *Phytochemistry* 62:691-706.

Ertl, D. S., Young, K. A., and Raboy, V. 1998. Plant genetic approaches to phosphorus management in agricultural production. *J. Environ. Qual.* 27:299-304.

Guttieri, M., Bowen, D., Dorsch, J. A., Raboy, V., and Souza E. 2004. Identification and characterization of a low phytic acid wheat. *Crop Sci.* 44:418-424.

Hitz, W. D., Carlson, T. J., Kerr, P. S., and Sebastian, S. A. 2002. Biochemical and molecular characterization of a mutation that confers a decreased raffinose and phytic acid phenotype on soybean seeds. *Plant Physiol.* 128:650-660.

Jang, D. A., Fadel, J. G., Klasing, K. C., Mireles, A. J., Ernst, R. A., Young, K. A., Cook, A., and Raboy, V. 2003. Evaluation of low-phytate corn and barley on broiler chick performance. *Poult. Sci.* 82:1914-1924.

Larson, S. R., Young, K. A., Cook, A., Blake, T. K., and Raboy, V. 1998. Linkage mapping of two mutations that reduce phytic acid content of barley grain. *Theor. Appl. Genet.* 97:141-146.

Leytem, A. B., Turner, B. L., and Thacker, P. A. 2004. Phosphorus composition of manure from swine fed low-phytate grains: Evidence for hydrolysis in the animal. *J. Environ. Qual.* 33:2380-2383.

Li, Y. C., Ledoux, D. R., Veum, T. L., Raboy, V., and Zyla, K. 2001a. Low phytic acid barley improved performance, bone mineralization, and phosphorus retention in turkey poults. *J. Appl. Poult. Res.* 10:178-185.

Li, Y. C., Ledoux, D. R., Veum, T. L., Raboy, V., Zyla, K., and Wikiera, A. 2001b. Bioavailability of phosphorus in low phytic acid barley. *J. Appl. Poultry Res.* 10:86-91.

Lott, J. N. A., Ockenden, I., Raboy, V., and Batten, G. D. 2000. Phytic acid and phosphorus in crop seeds and fruits: A global estimate. *Seed Sci. Res.* 10:11-33.

NRC. 1998. National Research Council. Nutritional Requirements of Swine. 10th Ed. National Academy Press: Washington, DC.

Overturf, K., Raboy, V., Cheng, Z. J., and Hardy, R. W. 2003. Mineral availability from barley *low phytic acid* grains in rainbow trout (*Oncorhynchus mykiss*) diets. *Aquaculture Nutr.* 9:239-246.

Raboy, V. 1997. Accumulation and storage of phosphate and minerals. Pages 441-477 in: Cellular and Molecular Biology of Plant Seed Development. B. A. Larkins and I. K. Vasil, eds. Kluwer: The Netherlands.

Raboy, V., Gerbasi, P. F., Young, K. A., Stoneberg, S. D., Pickett, S. G., Bauman, A. T., Murthy, P. P. N., Sheridan, W. F., and Ertl, D. S. 2000. Origin and seed phenotype of maize *low phytic acid 1-1* and *low phytic acid 2-1*. *Plant Physiol.* 124:355-368.

Raboy, V., Young, K. A., Dorsch, J. A., and Cook, A. 2001. Genetics and breeding of seed phosphorus and phytic acid. *J. Plant Physiol.* 158:489-497.

Rasmussen, S. K., and Hatzack, F. 1998. Identification of two low-phytate barley (*Hordeum vulgare* L.) grain mutants by TLC and genetic analysis. *Hereditas* 129:107-112.

Ravindran, V., Bryden, W. L., and Kornegay, E. T. 1995. Phytates: Occurrence, bioavailability, and implications in poultry nutrition. *Poult. Avian Biol. Rev.* 6:125-143.

Roslinsky, V. 2002. The development of molecular markers for low phytic acid mutants in barley (*Hordeum vulgare* L.). MS thesis. University of Saskatchewan: Saskatoon.

Sharpley, A. N., Daniel, T., Sims, J., Lemunyon, T., Stevens, R., and Parry, R. 2003. Agricultural phosphorus and eutrophication. 2nd Ed. Publication ARS-149. USDA-ARS: University Park, PA.

Spencer, J. D., Allee, G. L., and Sauber, T. E. 2000. Growth-finishing performance and carcass characteristics of pigs fed normal and genetically modified low-phytate corn. *J. Anim. Sci.* 78:1529-1536.

Thacker, P. A., Rossnagel, B. G., and Raboy, V. 2003. Phosphorus digestibility in low-phytate barley fed to finishing pigs. *Can. J. Anim. Sci.* 83:101-104.

Veum, T. L., Ledoux, D. R., Bollinger, D. W., Raboy, V., and Cook, A. 2002. Low-phytic acid barley improved calcium and phosphorus utilization and growth performance in growing pigs. *J. Anim. Sci.* 80:2663-2670.

Wilcox, J. R., Premachandra, G. S., Young, K. A., and Raboy, V. 2000. Isolation of high seed inorganic P, low-phytate soybean mutant. *Crop Sci.* 40:1601-160.

[Received December 27, 2005. Accepted April 24, 2006.]