Supplementary Information

Supplementary Methods: Table S1

Seed of the “two-rowed” malting barley cv. Harrington [1] were treated with sodium azide using a modification of the method as described [2], and advanced to the M2 generation. M3 seed sampled from a total of 2601 M2s were screened with the single-seed “high inorganic P” (HIP) test as described [3]. Wild-type barley seeds typically contain ≤0.7 mg inorganic P·g⁻¹. Seed testing for >1.0 mg inorganic P·g⁻¹ were deemed “HIP”. Direct-descendant lines (M6 to M8) that appeared homozygous for selected putative mutants were planted in a summer nursery in 2004 at the University of Idaho’s Aberdeen Research and Extension Center. Non-mutagenized cv. Harrington was included as a control. Seeds were harvested from individual plants and stored at 6 °C and 25% relative humidity. Subsequent analyses revealed that of the 23 putative mutants, one was a false positive and two (MAz104 and MAz237), while apparently heritable, had not yet been obtained as homozygotes. The remaining 20 were the subject of further analyses.

Two plants (replicates) representing each line were selected for analysis, and each replicate was analyzed in duplicate. Seed P fractions were determined in whole-seed samples, and following a simple dissection [4], in “germ-half-seeds” and “endosperm-half-seeds”. For whole-seed analyses, samples of 50 to 100 mature seeds were dried for 48 h at 60 °C and weighed. For half-seed analyses, samples of 20 seeds were cut in half by hand at the mid-way point between base and apex, the basal half containing the germ, and the apical half largely consisting of endosperm and aleurone. Whole-seed and half-seed samples were milled to pass through a 20-mesh (~0.853 mm) screen, and stored in a desiccator until analysis. Seed total P was determined following wet-ashing of aliquots of tissue (typically 150 mg) and colorimetric assay of digest P [3]. The ferric-precipitation method was used to determine total, acid-soluble inositol phosphates, referred to here as phytic acid P [5]. HPLC analyses indicated that in most genotypes >90% of total seed inositol phosphate consists of phytic acid (inositol hexakisphosphate), with the remainder consisting of less highly phosphorylated inositol phosphates such as inositol tetrakisphosphate or pentakisphosphate (data not shown). Thus use of the term “phytic acid P” for total inositol Ins phosphate is accurate in most cases. Aliquots of tissue (typically 0.5 to 1.0 gm) were extracted in 0.4 M HCl:0.7 M Na₂SO₄. Phytic acid P was obtained as a ferric precipitate, wet-ashed and assayed for P as in the total P analysis. Phytic acid P is expressed in terms of its P (atomic weight 31) content to facilitate comparisons between seed P fractions. Seed inorganic P was determined colorimetrically following extraction of tissue samples (typically 0.5 g in wild-type seeds and 0.15 g in mutant seeds) in 12.5% (w/v) TCA:25 mM MgCl₂. Statistical analyses were conducted using Statistical Analysis Systems (SAS) Software 9.2, SAS Institute Inc., Cary, NC, USA.
Table S1. Seed dry weight and seed phosphorus (P) fractions in 21 barley lines consisting of the wild-type control (cv. Harrington) and twenty mutants that display the “high inorganic P” seed phenotype. Seed was obtained from the 2004 Aberdeen, Idaho nursery. Please see Supplementary Methods for details of whole-grain and “half-seed” analysis and other methods used.

| Cultivar or Mutant Line | Seed Dry Weight | Total P | Phytic Acid P | PAP/Inorganic P | (PAP + Inorg. P)/Total P |
|-------------------------|----------------|---------|---------------|----------------|-------------------------|
|                         | mg· seed\(^{-1}\) | mg· g\(^{-1}\) | mg· g\(^{-1}\) | mg· g\(^{-1}\) |                        |
| Wild-Type               | 52.9           | 4.54    | 4.04          | 4.50           | 0.90                    |
|                         |                |         | Endo-Sperm    | Germ           | Endo/Germ               |
|                         |                |         | mg· g\(^{-1}\) | mg· g\(^{-1}\) |                        |
|                         | Whole          | Half-Seed Analysis | Whole          | Half-Seed Analysis |                        |
|                         | Grain         |           | Grain         |               |                         |
|                         | 2.75          | 2.73    | 3.07          | 0.89          | 0.60                    |
|                         |               |         |               |               |                         |
|                         |               |         |               |               |                         |
|                         |               |         |               |               |                         |
| Chromosome 2H Linkage Group |
| Hvlpa-1 (M422)           | 43.9          | 3.70    | 3.08          | 4.38           | 0.70                    |
| M281                    | 41.6          | 3.77    | 2.67          | 3.88           | 0.69                    |
| M882                    | 47.4          | 3.84    | 2.81          | 4.14           | 0.68                    |
| M1154                   | 45.7          | 3.70    | 2.66          | 4.24           | 0.63                    |
| M1580                   | 43.8          | 3.97    | 3.05          | 4.94           | 0.62                    |
| M1673                   | 43.9          | 3.72    | 2.87          | 4.38           | 0.66                    |
| M2002                   | 45.8          | 4.02    | 2.59          | 4.37           | 0.59                    |
| M2172                   | 42.6          | 3.68    | 2.54          | 4.20           | 0.60                    |
| Chromosome 7H Linkage Group |
| Hvlpa-2 (M1070)          | 46.5          | 4.85    | 4.38          | 4.69           | 0.93                    |
| M640                    | 38.9          | 4.19    | 3.13          | 4.98           | 0.63                    |
| Chromosome 1H Linkage Group |
| Hvlpa-3 (M635)           | 43.5          | 4.80    | 4.14          | 4.64           | 0.89                    |
| M955                    | 39.4          | 5.00    | 4.62          | 5.17           | 0.89                    |
Table S1. Cont.

| Cultivar or Mutant Line | Seed Dry Weight |   |   |   |   |   |   |   |   |   |   |
|-------------------------|-----------------|---|---|---|---|---|---|---|---|---|---|
|                         | Whole Grain mg· seed⁻¹ |  |   |   |   |   |   |   |   |   |   |
|                         | Half-Seed Analysis mg· g⁻¹ |  |   |   |   |   |   |   |   |   |   |
|                         | Endo-Sperm | Germ | Endo/ Germ |   |   |   |   |   |   |   |
|                         | mg· g⁻¹ | mg· g⁻¹ |   |   |   |   |   |   |   |   |
| Hvlpa-1 (M593)          | 46.9 | 4.57 | 3.80 | 4.26 | 0.89 | 1.28 | 0.94 | 1.29 | 0.73 | 0.28 | 2.18 |
|                         | 29.2 | 5.22 | 4.78 | 5.32 | 0.90 | 0.18 | 0.13 | 0.18 | 0.75 | 0.04 | 3.76 |
|                         | 52.2 | 3.84 | 3.53 | 3.63 | 0.98 | 1.70 | 1.35 | 1.59 | 0.84 | 0.44 | 1.00 |
|                         | 51.4 | 4.75 | 4.54 | 5.19 | 0.88 | 2.14 | 1.54 | 1.78 | 0.87 | 0.45 | 1.90 |
|                         | 46.8 | 4.73 | 4.08 | 4.32 | 0.94 | 2.40 | 2.28 | 2.19 | 1.04 | 0.51 | 1.29 |
| M499                    | 44.4 | 4.77 | 4.05 | 4.54 | 0.89 | 2.40 | 2.33 | 2.34 | 1.00 | 0.51 | 1.08 |
| M2080                   | 38.8 | 4.70 | 4.43 | 4.41 | 1.00 | 1.93 | 1.91 | 1.66 | 1.15 | 0.41 | 1.71 |
| MAZ423                  | 49.2 | 5.61 | 4.94 | 5.50 | 0.90 | 3.17 | 2.99 | 3.14 | 0.95 | 0.56 | 1.12 |
| LSD 0.05                | 7.3  | 0.47 | 0.50 | 0.59 | 0.07 | 0.40 | 0.21 | 0.24 | 0.09 | 0.09 | 0.38 |
| Others Mutants of presently unknown inheritance and map position. |   |   |   |   |   |   |   |   |   |   |   |
| M499                    | 44.4 | 4.77 | 4.05 | 4.54 | 0.89 | 2.40 | 2.33 | 2.34 | 1.00 | 0.51 | 1.08 |
| M2080                   | 38.8 | 4.70 | 4.43 | 4.41 | 1.00 | 1.93 | 1.91 | 1.66 | 1.15 | 0.41 | 1.71 |
| MAZ423                  | 49.2 | 5.61 | 4.94 | 5.50 | 0.90 | 3.17 | 2.99 | 3.14 | 0.95 | 0.56 | 1.12 |
| LSD 0.05                | 7.3  | 0.47 | 0.50 | 0.59 | 0.07 | 0.40 | 0.21 | 0.24 | 0.09 | 0.09 | 0.38 |
| F Value                 | 4.55 *** | 13.2 *** | 22.3 *** | 5.63 *** | 39.3 *** | 28.3 *** | 142 *** | 80.6 *** | 104 *** | 21.3 *** | 38.6 *** |

Chromosome 4H Linkage Group

| Cultivar or Mutant Line | PAP/ Tot P mg· g⁻¹ | Inorganic P mg· g⁻¹ | (PAP + Inorg. P)/ Total P mg· g⁻¹ |
|-------------------------|------------------|---------------------|---------------------------------|
| Hvlpa-1 (M593)          | 0.76             | 0.76                | 0.76                            |
| M678                    | 0.76             | 0.76                | 0.76                            |
| M889                    | 0.70             | 0.70                | 0.70                            |
| M1572                   | 0.85             | 0.85                | 0.85                            |
| M1954                   | 0.78             | 0.78                | 0.78                            |
| M499                    | 0.73             | 0.73                | 0.73                            |
| M2080                   | 0.77             | 0.77                | 0.77                            |
| MAZ423                  | 0.76             | 0.76                | 0.76                            |
| LSD 0.05                | NA               | NA                  | NA                              |
| F Value                 | 0.80 NS          | 0.80 NS             | 0.80 NS                         |
The barley cultivars “Morex” [6] and “Steptoe” [7] are widely used in barley mapping and genomics research and represent distinct germplasm pools as compared with cv. Harrington [8]. Therefore for chromosomal mapping, \( F_2 \) populations were derived from crosses between each Harrington-derived seed P mutant and both Morex and Steptoe. \( F_2 \)'s were grown at the Aberdeen Research and Extension Center in 2002, and in subsequent greenhouse nurseries, to provide populations ranging in number from approximately 100 to 200 recombinants. \( F_3 \) progeny seed for each \( F_2 \) plant were harvested and stored until analysis. 

Segregation analysis for each seed P mutation was conducted by testing individual \( F_3 \) seed for the “high inorganic P” typical of each mutation. As homozygotes these mutations condition increases in seed inorganic P as compared with a heterozygote or homozygous wild-type seed that are typically large enough to permit clear-cut scoring on an individual kernel basis [9,10]. Typically a minimum of 30 individual kernels from each \( F_3 \) progeny were tested. If all or nearly all (>27) \( F_3 \) seed displayed the seed inorganic P phenotype typical of the parental mutant, the \( F_2 \) parent plant was scored as homozygous mutant. If all \( F_3 \) seed were scored as wild-type, then the \( F_2 \) parent plant was scored as homozygous wild-type. If segregation for the HIP phenotype was observed in the \( F_3 \) (three or more kernels per 30 \( F_3 \) kernels scored as mutant/high inorganic P, with the remainder intermediate or wild-type/low inorganic P), then the parent \( F_2 \) were scored as heterozygotes. If the result of the initial round of seed testing was inconclusive, additional \( F_3 \) seed testing was conducted. Data were collected using either images of colorimetric reactions or digital readings from Synergy™ 2 Multi-Detection Microplate Reader (BioTEK, Winooski, VT, USA). For mapping purposes, \( F_{2-3} \) families homozygous for either mutant or wild-type alleles from each segregating population were used to simplify the mapping score. Leaf samples were collected, immediately frozen in liquid N\(_2\) and stored at −80 °C.

PCR-based markers linked to each of the three previously-known Hv\textit{lp}a loci and the barley genome’s single gene encoding \( \text{myo-Inositol-3-P}_1 \) synthase (MIPS) [11] were selected based on the previously published data [9–11]. For the \( \text{Hv}lpa1 \) locus on barley chromosome 2H, aMSU21 was identified as a closely linked STS-PCR marker [9,12]. New primer pairs for the aMSU21 locus were designed; aMSU21 Forward (5’-tggtctttcatgtacctacc-3’) and aMSU21 Reverse (5’-tggtcatcaagcaacaac-3’). The newly designed primer pairs amplify a single strong 435 bp PCR product from Steptoe and Morex that is slightly shorter than the original 449 bp product, and about 200 bp bigger PCR products from Harrington and its derived mutations. The original primer pairs of aMSU21 detected two major fragments from each cultivar [12] while our modified primer pairs detected one major fragment from each genotype (data not shown). Thus the modified marker simplified the genotype scoring. The barley MIPS locus on chromosome 4H was first mapped close to the BCD453 marker [11]. The EBmac701 SSR marker was identified as flanking BCD453B (Barley Consensus Map 2005, GrainGenes, http://wheat.pw.usda.gov/cgi-bin/graingenes/report.cgi?class=mapdata&name=Barley,+Consensus,+2005,+SNP) [11]. The EBmac701 primer pairs listed in GrainGenes (Forward, 5’-atgatgaactcttcacc-3’ and Reverse, 5’-tggtcatcaagcaacaac-3’) amplified a single strong PCR product. The polymorphic patterns were clearly scored as a ~150 bp product in Harrington and slightly smaller, but clearly scorable fragments in Steptoe and Morex (data not shown).
For the Hvlp2 locus on barley chromosome 7H, the previously described flanking RFLP markers were MWG2301 and ABC310b [9]. Bmag0120 was chosen based on its close location (Barley shedar2 map, Grain Genes) to ABC310b that was loosely linked to Hvlp2-1 [9]. The Bmag120 primers listed in GrainGenes (Forward, 5’-attctacccaaagagac-3’ and Reverse, 5’-gtcacatagagttctcc-3’) amplified a ~250 bp single product in Harrington and a slightly smaller product in Steptoe. For the Hvlp3 locus on barley chromosome 1H, the RFLP probes cWMG706 and ABG702B were shown to flank the locus and the closely-linked LP75 sequence-specific ISSR primer was developed [10]. Since LP75 requires ClaI digestion of PCR products to detect polymorphism, the Bmag382 SSR marker, closely linked to cWMG706 (lgri x Franka map, GraineGenes) was chosen for this experiment. The Bmag382 primer pairs listed in GrainGenes (Forward, 5’-tgaaacccatagagtaga-3’ and Reverse, 5’-tcacaagtctgttcaata-3’) amplified a strong single PCR product in all barley cultivars in this experiment. The PCR product from Harrington is slightly above the 100 bp size marker distinguishing the slightly smaller fragments from Steptoe and Morex (data not shown).

Leaf samples collected from F2–3 plants were lyophilized prior to DNA extraction with cetyl trimethyl ammonium bromide (CTAB). Pulverized leaf tissue in a 96-well format was extracted with the protocol utilized by [13]. The only modification to the procedure was the use of chloroform without isoamyl alcohol.

PCR reactions were set up in a 96-well format. Each 25 μL reaction contained 50 ng of template DNA, 2 μL of each primer (10 μM), 2.5 μL of 10× buffer containing 1.1 mM Mg, 1 μL of dNTPs with 2.5 mM concentration for each nucleotide, and 1 unit of Taq polymerase (RedTaq, Sigma). The PCR program was: 94 °C for 3 min followed by 39 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min, followed by a 4 °C hold. PCR reaction products were analyzed on 3% Agarose SFR high resolution gels (Amresco, Solon, OH, USA) stained with ethidium bromide. A 100 bp DNA ladder (Bio-Rad, Richmond, CA, USA) was used as a size marker in the same gel.

The PCR fragments were scored for each individual F2 plant from segregating populations and compared to the genotype patterns of both parents. Mutant or wild-type scores were recorded for all the plants used. The genotype scores were then compared to the seed inorganic P phenotype as determined in F3 seeds derived from F2 plants described above.
Table S2. Segregation ratios observed in barley low phytic acid (lpa) F₂ mapping populations. Please see Supplementary Methods for details of analyses.

| Parental Cross | F₂ Progeny Genotypes \(^{a}\) | Chi-Square \((1:2:1)^{b}\) | Chi-Square \((3:1)^{c}\) |
|----------------|---------------------------------|----------------------------|----------------------------|
|                | \(Lpa/Lpa\) | \(Lpa/lpa\) | \(lpa/lpa\) | \(Lpa/Lpa\) | \(Lpa/lpa\) | \(lpa/lpa\) | \(Lpa/Lpa\) | \(Lpa/lpa\) | \(lpa/lpa\) | \(Lpa/Lpa\) | \(Lpa/lpa\) | \(lpa/lpa\) | \(Lpa/Lpa\) | \(Lpa/lpa\) | \(lpa/lpa\) | \(Lpa/Lpa\) | \(Lpa/lpa\) | \(lpa/lpa\) | \(Lpa/Lpa\) | \(Lpa/lpa\) | \(lpa/lpa\) |
| M 281 × Steptoe | 17 | 36 | 19 | 0.2 | 0.1 |
| M 499 × Steptoe | 16 | 22 | 4 | 6.9 * | 5.3 * |
| M 593 × Steptoe | 22 | 42 | 23 | 0.3 | 0.2 |
| M 640 × Steptoe | 22 | 44 | 16 | 1.3 | 1.3 |
| M 678 × Steptoe | 22 | 31 | 19 | 1.7 | 0.1 |
| M 882 × Steptoe | 22 | 51 | 23 | 0.6 | 0.2 |
| M 889 × Morex | 45 | 33 | 5 | 42 * | 15.9 * |
| M 1154 × Steptoe | 24 | 49 | 20 | 0.6 | 0.7 |
| M 1572 × Steptoe | 24 | 42 | 19 | 0.8 | 0.6 |
| M 1580 × Steptoe | 23 | 34 | 28 | 3.9 | 2.8 |
| M 1673 × Steptoe | 26 | 42 | 19 | 2.7 | 1.8 |
| M 1954 × Steptoe | 23 | 35 | 10 | 5.0 | 3.9 * |
| M 2002 × Steptoe | 12 | 45 | 10 | 8.0 * | 3.7 |
| M 2172 × Morex | 28 | 40 | 8 | 10.8 * | 7.8 * |

* Significant deviation from expected genotype ratio (\(\alpha = 0.05\)); \(^{a}\) \(Lpa/Lpa\), \(Lpa/lpa\), and \(lpa/lpa\) indicates homozygous wild-type, heterozygous and homozygous mutant, respectively; \(^{b}\) Critical value for Chi-square distribution with tail area probability of 0.05 and \(df = 2\) is 5.99; \(^{c}\) Critical value for Chi-square distribution with tail area probability of 0.05 and \(df = 1\) is 3.84.

Table S3. Linkage analyses of SSR markers to barley low phytic acid mutations. Please see Supplementary Methods for details of analysis.

| Mutation | DNA Marker Linkage |
|----------|--------------------|
|          | aMSU21 | EBmac701 | Bmag0120 | Bmag382 |
|          | Rec. \(/\) Total | Genetic Distance (cM) | Rec. \(/\) Total | Genetic Distance (cM) | Rec. \(/\) Total | Genetic Distance (cM) | Rec. \(/\) Total | Genetic Distance (cM) |
| M 281 | 3/160 | 1.9 | 26/51 | 51.0 | 8/51 | 15.7 | 27/51 | 52.9 |
| Hvlda1-1 | 8/142 | 5.6 | 12/41 | 29.3 | 18/57 | 31.6 | 33/57 | 41/57 |
| M 882 | 1/41 | 2.4 | 2/42 | 4.8 | 2/58 | 3.4 | 1/51 | 2.0 |
| M 1154 | 2/42 | 4.8 | 2/58 | 3.4 | 1/51 | 2.0 | 1/138 | 1.0 |
| M 1580 | 2/42 | 4.8 | 2/58 | 3.4 | 1/51 | 2.0 | 1/138 | 1.0 |
| M 1673 | 1/51 | 2.0 | 12/51 | 51.0 | 8/51 | 15.7 | 27/51 | 52.9 |
| M 2002 | 1/138 | 1.0 | 12/51 | 51.0 | 8/51 | 15.7 | 27/51 | 52.9 |
| M 2172 | 1/63 | 2.0 | 12/51 | 51.0 | 8/51 | 15.7 | 27/51 | 52.9 |
| M 640 | 28/51 | 54.9 | 12/51 | 51.0 | 8/51 | 15.7 | 27/51 | 52.9 |
| M 1954 | 12/51 | 29.3 | 12/51 | 51.0 | 8/51 | 15.7 | 27/51 | 52.9 |
| M 593 | 18/57 | 31.6 | 18/57 | 31.6 | 33/57 | 41/57 | 20/34 | 58.8 |
| M 1572 | 12/50 | 22.0 | 12/50 | 22.0 | 33/57 | 41/57 | 20/34 | 58.8 |
| M 889 | 25/34 | 73.5 | 10/34 | 29.4 | 10/34 | 29.4 | 20/34 | 58.8 |
| M 678 | 46/66 | 69.7 | 46/66 | 69.7 | 47/66 | 71.2 | 47/66 | 71.2 |
| M 499 | 22/28 | 78.6 | 17/28 | 60.7 | 16/28 | 57.1 | 17/28 | 60.7 |

* Rec. = recombinants in the tested population. The raw scored data is presented here.
**Supplementary Methods: Figure S1**

Forty seed were randomly sampled from the seed bulk representing each isoline. Individual seed were crushed with a lab press, extracted overnight in 250 μL 0.4 M HCl, and 10 μL extract assayed for seed inorganic P using a microtitre plate assay as described [5]. The Colorimeric Standards No. 1 through 5 contain 0.0, 0.15, 0.46, 0.93, and 1.39 μg inorganic P per well. Wild-type barley seed typically contain ≤0.5 mg inorganic P·g⁻¹ dry weight, and single-seed assays usually result in tests visually similar to Standards 1 through 3. Homozygous lpa seed typically contain ≥1.0 mg inorganic P·g⁻¹, and single-seed assays usually result in tests similar to Standards 4 and 5.

**Figure S1.** Tests for homozygosity and homogeneity in barley *low phytic acid* near-isogenic lines. Single-seed tests were conducted for inorganic P in seed produced by pairs of barley near-isogenic lines, each pair consisting of a sibling wild-type or homozygous mutant line. The Colorimeric Standards No. 1 through 5 contain 0.0, 0.15, 0.46, 0.93, and 1.39 μg inorganic P per well. Wild-type barley seed typically contain ≤0.5 mg inorganic P·g⁻¹ dry weight, and single-seed assays usually result in tests visually similar to Standards 1 through 3. Homozygous lpa seed typically contain ≥1.0 mg inorganic P·g⁻¹, and single-seed assays usually result in tests similar to Standards 4 and 5.
Supplementary References

1. Harvey, B.L.; Rossnagel, B.G. Harrington barley. *Can. J. Plant Sci.* 1984, 64, 193–194.
2. Nilan, R.A.; Sideris, E.G.; Sander, C.; Konzak, C.F. Azide—A potent mutagen. *Mutat. Res.* 1973, 17, 142–144.
3. Dorsch, A.J.; Cook, A.; Young, A.K.; Anderson, N.J.; Bauman, T.A.; Volkmann, J.C.; Murthy, P.N.P.; Raboy, V. Seed phosphorus and inositol phosphate phenotype of barley low phytic acid genotypes. *Phytochemistry* 2003, 62, 691–706.
4. Raboy, V.; Cichy, K.; Peterson, K.; Reichman, S.; Sompong, U.; Srinives, P.; Saneoka, H. Barley (*Hordeum vulgare L.*) low phytic acid 1-1: An endosperm-specific determinant of seed total phosphorus. *J. Hered.* 2014, 105, 656–665.
5. Raboy, V.; Gerbasi, P.F.; Young, K.A.; Stoneberg, S.D.; Pickett, S.G.; Bauman, A.T.; Murthy, P.P.N.; Sheridan, W.F.; Ertl, D.S. Origin and seed phenotype of maize low phytic acid 1-1 and low phytic acid 2-1. *Plant Physiol.* 2000, 124, 355–368.
6. Rasmusson, D.C.; Wilcoxson, R.D. Registration of Morex barley. *Crop Sci.* 1979, 19, 293.
7. Muir, C.E.; Nilan, R.A. Registration of Steptoe barley. *Crop Sci.* 1973, 13, 770.
8. Matus, I.A.; Hayes, P.M. Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. *Genome* 2002, 44, 1095-1106.
9. Larson, S.R.; Young, K.E.; Cook, A.; Blake, T.K.; Raboy, V. Linkage mapping two mutations that reduce phytic acid content of barley grain. *Theor. Appl. Genet.* 1998, 12, 141–146.
10. Roslinsky, V.; Eckstein, P.E.; Raboy, V.; Rossnagel, B.G.; Scoles, G.J. Molecular marker development and linkage analysis of low phytic acid barley (*Hordeum vulgare*) mutant lines. *Mol. Breeding* 2007, 20, 323–330.
11. Larson, S.R.; Raboy, V. Linkage mapping of maize and barley myo-inositol 1-phosphate synthase DNA sequences: Correspondence with a low phytic acid mutation. *Theor. Appl. Genet.* 1999, 99, 27–36.
12. Shin, J.S.; Chao, S.; Corpuz, L.; Blake, T.K. A partial map of the barley genome incorporating restriction fragment length polymorphism, polymerase chain reaction, isozyme, and morphological marker loci. *Genome* 1990, 33, 803–810.
13. Jackson, E.W.; Avant, J.B.; Oerturf, K.E.; Bonman, J.M. A quantitative assay of *Puccinia coronata* f. sp. *avena* DNA in *Avena sativa*. *Plant Dis.* 2006, 90, 629–636.