# Near-Infrared Reflectance Correlated to 100-g Wet-Milling Analysis in Maize

A. Dijkhuizen,[1] J. W. Dudley,[1,2] T. R. Rocheford,[1] A. E Haken,[3] and S. R. Eckhoff[3]

## ABSTRACT

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An understanding of the genetic control of starch, protein, and oil concentrations in the corn (*Zea mays* L.) kernel is essential for improvement of grain quality. Because large numbers of progenies are needed for genetic studies, a rapid, accurate, analytical procedure is necessary. As part of a study to identify chromosomal regions associated with starch and protein, a rapid near-infrared reflectance (NIR) method and a more labor-intensive 100-g wet-milling procedure were compared for consistency in ranking families and identifying quantitative trait loci (QTL) using a set of 200 $F_2S_1$ families from the cross of the 70th generations of the Illinois High Protein (IHP) × Illinois Low Protein (ILP) corn strains. NIR starch and wet-milling starch values were highly correlated ($r$ = 0.80), as were NIR protein and gluten measured by wet-milling ($r$ = 0.72). Chromosomal regions associated with NIR starch and wet-milling starch were generally the same. Fiber concentration was significantly negatively correlated with starch and positively correlated with protein. Chromosome regions with significant associations with starch also had significant associations with fiber. The NIR method is satisfactory for measuring starch and protein in material with a wide range of variability in the early stages of a corn-breeding program.

Historically, the majority of U.S. corn has been grown for animal feed. However, the fastest growing use of corn today is for food and industrial uses. Most of the growth in industrial use of corn has been in the area of wet-milling, which currently accounts for 75% of processed corn (Eckhoff 1992). Of products obtained from wet-milling, starch is the one of chief importance. Corn starch can be converted into a wide variety of end products, including paper, sweeteners, industrial chemicals, and ethanol, a renewable source of energy.

One of the requirements for corn starch to be competitive with other sources for the various end products is availability of high-yielding corn hybrids with a high starch concentration. The Illinois long-term selection strains divergently selected for protein and oil concentration represent extremes in terms of protein, oil, and starch concentration. As a result, these strains are ideal for studying chemical composition of the corn kernel. Recently, a number of studies have been conducted to identify and study chromosomal regions affecting starch, oil, and protein concentration (Goldman et al 1993, 1994; Berke and Rocheford 1995) with the intention of using this information to more efficiently manipulate these traits. These studies have shown that chromosome regions (quantitative trait loci [QTL]) that affect starch concentration in the corn kernel can be identified.

All QTL studies involving the protein strains have relied on measurement of kernel composition traits by near-infrared reflectance (NIR) using a Dickey-john GAC III NIR analyzer (Hymowitz et al 1974, Dudley and Lambert 1992). NIR measurements are based on reflectance of a ground whole kernel corn meal sample at six different wavelengths.

The wet-milling procedure relies on physical (e.g., hardness) and chemical (e.g., solubility) properties of the various tissues making up the corn kernel to separate it into physical and chemical components. As a result, not only the concentration of individual components, but also the location and chemical makeup of each of the chemical products is important. The chemical composition in one of the tissues (e.g., oil concentration in the germ) may affect the physical properties of the tissue, and thus the extractability. Therefore, differences in trait expression based on NIR measurements may not result in differences in extractability of a specific constituent using wet-milling analysis and vice versa.

In the past, costs and labor requirements for wet-milling analysis prohibited evaluation of large numbers of samples. The recent development of a 100-g wet-milling procedure by Eckhoff et al (1996) has greatly reduced the cost of a single analysis and increased the number of samples that can be analyzed as compared to procedures described previously. This has allowed comparison of the NIR and the wet-milling procedures using a population large enough to conduct QTL analysis.

The objectives of this study were to: 1) test the level of agreement between NIR and wet-milling performance, and 2) identify regions of the genome associated with each chemical component and wet-milling fraction.

## MATERIALS AND METHODS

Parental populations used in this experiment were the 70th generation of the Illinois High Protein (IHP) and Illinois Low Protein (ILP) strains from the Illinois long-term selection experiment (Dudley and Lambert 1992). Mapping populations were developed from a strain cross between generations 70 of IHP (44% extractable starch, 26.1% protein [N × 6.25], 5.4% oil) and ILP (74.5% extractable starch, 5.8% protein, 4.2% oil) (Dudley et al 1977, Dudley and Lambert 1992). The initial mating involved five to seven plants of each of the parental strains. $F_1$ plants were intermated to obtain an $F_2$ bulk. To obtain $F_2S_1$ families, 200 individuals were self-pollinated.

### Phenotypic Evaluation

The 200 $F_2S_1$ families were evaluated at the Crop Sciences Research and Education Center, Urbana, IL, in 1995, using two planting dates (environments). Within each environment, families were arranged in a generalized lattice (α [0,1]) design with two replicates of 20 incomplete blocks of 10 plots each (Carmer 1985). The IHP and ILP parental populations were evaluated in an adjacent experiment in each of the two environments using a randomized complete block design with 20 replicates. Planting dates were May 3 and May 30, 1995. Plots consisted of a single 4.57-m row with 0.76 m between rows. Plots were over-seeded and thinned at the four- to seven-leaf stage to 15 plants per row. In each plot, six plant-to-plant crosses were made. Controlled pollinations were used to obtain kernel composition estimates unaffected by gametes from neighboring plots.

### Chemical Analyses

*Method 1, NIR.* Seed from hand-pollinated ears within a plot was bulked, and kernel starch, protein, and oil concentrations were measured on a per-plot basis using the bulked seed. Starch, protein, and oil concentrations were measured using a Dickey-john GAC III NIR analyzer (Hymowitz et al 1974) as outlined by

[1] Department of Crop Sciences, University of Illinois, Urbana 61801.
[2] Corresponding author. Phone: 217/333-9640. E-mail: jdudley@uiuc.edu
[3] Department of Agricultural Engineering, University of Illinois, Urbana IL 61801.

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Dudley and Lambert (1992). The machine was calibrated using a set of standard samples with known kernel composition representing the maximum range of possible concentrations. Using reflectance values from the standard samples, a multiple regression equation was developed and used to predict values from samples of unknown concentration. Results are expressed as the concentration of each of the chemical components on a dry matter basis. To allow for comparison of wet-milling and NIR values, NIR $S_1$ and parental strain means were calculated for each environment using the PROC MIXED procedure (SAS Institute Inc., Cary, NC) adjusting for replicate and block-within-replicate effects.

*Method 2, 100-g wet-milling analysis.* For wet-milling analysis, seed from two replicates of an $F_2S_1$ family within an environment was bulked. Thus, each entry was represented by two samples, one from each environment. For the parental IHP and ILP strains, seed from all 20 replicates within an environment was bulked. From each environment, two subsamples of each of the parental strains were taken, except for ILP for which only one subsample was taken in one of the environments. Thus, the mean for IHP is represented by four subsamples and the mean for ILP is represented by three subsamples. Composition of each subsample was determined using the 100-g wet-milling analysis as outlined by Eckhoff et al (1996). This procedure yields starch, gluten, fiber, germ, and steepwater fractions. Gluten contains a high concentration of protein, and the oil is contained in the germ. The steepwater solids fraction includes soluble components such as minerals, vitamins, soluble protein, and soluble sugars.

### Restriction Fragment Length Polymorphism (RFLP) Genotyping

Fresh tissue was bulked from 20 random plants per $S_1$ family and ground in liquid $N_2$. DNA in 5-g samples of finely ground leaf tissue frozen in fresh liquid $N_2$ was isolated using a modified cTAB procedure (Saghai-Maroof et al 1984, Hoisington 1991). DNA (1–15 μg) was digested with 25 units of *Eco*RI, *Eco*RV, or *Hin*dIII endonucleases and loaded on 8 g/L of agarose gel and size-fractionated at a field strength of 52 V/m for 18 hr. Southern blotting was done according to Hoisington (1991). DNA was transferred to 0.45-μm nylon filters and bound by UV linking and baking at 85°C for 2 hr.

Genomic clones were selected from collections of mapped maize clones developed and provided by the University of Missouri (Columbia, MO) (Umc), Brookhaven National Laboratories (Upton, NY) (Bnl), and Pioneer Hi-bred International (Johnston, IA) (Php). Clones from Pioneer also included a set of clones from Native Plants, Inc. (Salt Lake City, UT) (Npi). Also used were two cDNA clones, *Agr*r115, a core probe of the University of Missouri maize map, and *shrunken*-2 (*sh*2) (Bhave et al 1990), a cDNA clone of the large subunit of endosperm-specific ADP-glucose pyrophosporylase (AGP), an essential enzyme in starch biosynthesis. Genomic and cDNA clones were oligo-labeled (Feinberg and Vogelstein 1983) and hybridized to nylon filters according to Hoisington (1991). Initial emphasis was on probes and chromosome regions previously identified as affecting kernel composition from the cross of the 76th generations of IHP × ILP (Goldman et al 1993). Probes were selected based on polymorphism between the two parental populations, strong signals, and distribution across the maize genome. In total, 71 probes were used, including eight on chromosome 1; eight on chromosome 2; 14 on chromosome 3; five on chromosome 4; nine on chromosome 5; three on chromosome 7; seven on chromosome 8; five on chromosome 9; and seven on chromosome 10. Probe-allele combinations were scored as 1, 0, or –1 depending on homozygous presence, heterozygous presence, or absence of that allele.

### Statistical Analysis

Using the PROC MIXED procedure, variances due to $S_1$ family genotype ($\sigma^2_g$) and residual ($\sigma^2$) effects were estimated for each trait. Effects were tested and variance components were estimated fitting the model: $Y_{ij} = L_i + G_j + E_{ij}$, where $Y_{ij}$ is the phenotypic value of the $j$th family grown in the $i$th environment. The environment ($L$) was considered fixed, and $S_1$ family ($G$) was considered a random effect. $E_{ij}$ is a random error. Repeatabilities ($H^2$) were estimated as: $H^2 = \sigma^2_g/(\sigma^2_g + \sigma^2/2)$.

Phenotypic ($r_p$) and genotypic ($r_g$) correlations were calculated for all trait combinations. Phenotypic correlations were based on $S_1$ family means using the PROC CORR (SAS) procedure. Genotypic correlations were calculated according to Kempthorne (1957) using variance components obtained from the PROC MIXED procedure.

For each marker-allele combination, families were given a coded value of 1, 0, or –1 (described above). Marker alleles without at least 10 individuals in two of the three marker classes were excluded from QTL analyses. Because of the presence of multiple alleles, separate analyses were done for each allele as described by Zehr et al (1992). For each generation, family means averaged over replicates and environments were used to determine marker-QTL associations using linear regression based on marker class means. A marker-QTL association was declared significant if $P < 0.01$ using the $F$-test for linear effects. The slope of the regression of a trait mean on coded marker allele classes was used as an estimate of the average effect of allelic substitution.

## RESULTS AND DISCUSSION

### Phenotypic Evaluation

In 1995, precipitation was lower than average during most of the growing season. In addition to dry conditions, high temperatures caused visible plant stress during anthesis and grain fill.

**TABLE I**
**Sample Composition (% dry weight) as Determined by Near-Infrared Reflectance (NIR) and 100-g Wet-Milling Analysis for Cycle 70 of the Illinois High Protein (IHP) and Illinois Low Protein (ILP) Corn Strains and Their $F_2S_1$ Offspring**

| Generation | IHP | ILP | $F_2S_1$ (Mean) | $F_2S_1$ (Range) |
|---|---|---|---|---|
| 100-g wet-milling analysis | | | | |
| Starch | 39.0 ± 1.1 | 68.8 ± 1.4 | 57.9 ± 0.3 | 45.1–65.1 |
| Gluten | 24.1 ± 0.6 | 10.6 ± 0.7 | 16.1 ± 0.2 | 12.4–23.1 |
| Fiber | 26.3 ± 0.6 | 16.1± 0.6 | 17.7 ± 0.1 | 14.2–23.8 |
| Germ | 5.4 ± 0.3 | 1.7 ± 0.7 | 4.6 ± 0.1 | 2.4–7.5 |
| Steepwater solids | 4.7 ± 0.1 | 2.6 ± 0.1 | 3.5 ± 0.0 | 2.9–4.3 |
| Total | 99.5 ± 0.1 | 99.8 ± 0.1 | 99.8 ± 0.0 | 99.5–100.2 |
| NIR (% dry weight) | | | | |
| Starch | 40.6 ± 0.3 | 66.8 ± 0.5 | 60.4 ± 0.4 | 55.3–64.5 |
| Protein | 22.9 ± 0.3 | 5.9 ± 0.3 | 13.1 ± 0.2 | 10.4–16.3 |
| Oil | 5.5 ± 0.1 | 5.1 ± 0.1 | 5.3 ± 0.0 | 4.8–5.9 |
| Total | 69.0 ± 0.3 | 77.9 ± 0.4 | 78.7 ± 0.3 | 76.2–80.6 |

**TABLE II**
**Variance Components[a] and Repeatability[b] ($H^2$) for Corn Kernel Composition (% dry weight) Measured on 200 $F_2S_1$ Families Derived from the Illinois High Protein (IHP) and Illinois Low Protein (ILP) Corn Strains Mating Using Near-Infrared Reflectance (NIR) and 100-g Wet-Milling Analyses[c]**

| Trait | $\sigma^2_g$ | $\sigma^2$ | $H^2$ |
|---|---|---|---|
| 100-g wet-milling analysis | | | |
| Starch | 14.43 ± 1.68 | 37.50 ± 0.41 | 0.89 |
| Gluten | 3.79 ± 0.47 | 1.43 ± 0.16 | 0.84 |
| Fiber | 2.90 ± 0.35 | 0.89 ± 0.10 | 0.87 |
| Germ | 1.36 ± 0.16 | 0.36 ± 0.04 | 0.88 |
| Steepwater solids | 0.0790 ± 0.009 | 0.019 ± 0.002 | 0.89 |
| NIR | | | |
| Starch | 3.82 ± 0.47 | 1.53 ± 0.15 | 0.83 |
| Protein | 1.81 ± 0.22 | 0.63 ± 0.06 | 0.85 |
| Oil | 0.057 ± 0.008 | 0.034 ± 0.003 | 0.77 |

[a] $\sigma^2_g$ and $\sigma^2$ designate variance due to genotype and error, respectively.
[b] Repeatabilities ($H^2$) computed as $\sigma^2_g/(\sigma^2_g + \sigma^2/2)$.
[c] All effects were significant at $P < 0.0001$.

Parental strains had poor germination resulting in erratic stands and appeared less vigorous than most of the recombinant $S_1$ families. Lack of vigor was manifested in IHP and ILP as later flowering dates and lower kernel weight than those of the $S_1$ families (data not shown).

As expected, based on previous data (Dudley and Lambert 1992), IHP and ILP differed dramatically in protein and starch concentrations (Table I) as measured by NIR. Differences in NIR oil values were significant but much smaller than the differences in protein and starch. These differences were reflected in the ranges among the $S_1$ families. As in the NIR analysis, IHP and ILP differed markedly for wet-milling starch. However, the wet-milling starch values were higher for ILP and lower for IHP than were the NIR values.

The range in starch concentrations among $S_1$ families measured using NIR was narrower than it was for starch extracted with the wet-milling procedure (Table I). In the ILP strain, 2.0 percentage points more starch was isolated than was estimated based on NIR measurements. However, the upper limit for the $S_1$ families was only 0.6 percentage points higher for the wet-milling procedure than for NIR. The increased range in wet-milling starch among $F_2S_1$ families was primarily the result of lower starch values (10.2 percentage points) at the low end of the distribution. This may have resulted from interference of high protein in starch extraction. Alternatively, the NIR is calibrated based the chemical extraction procedure of method A-20 of the Corn Refiners Association (CRA 1980). If results from that procedure do not agree with the wet-milling procedure, the NIR values will not agree with it either.

Protein estimates according to NIR and gluten estimates from wet-milling analysis are very different, particularly for the ILP strain (Table I). The gluten concentration of ILP was 4.7 percentage points higher according to the wet-milling procedure than the protein estimate based on NIR. The mean of $F_2S_1$ families is 3.0 percentage points higher for gluten than for NIR protein and the range is greater. A possible explanation for the higher estimate based on wet-milling is that some of the gluten fraction may actually be oil from unrecovered brittle germ or starch that was not separated from the gluten. There is an inherent discrepancy between protein recovered using the wet-milling procedure and the estimate based on NIR. The NIR is calibrated based on protein measured using the Kjeldahl procedure. Thus, the water-soluble albumins, salt-soluble globulins, alcohol-soluble prolamins, and alkaline-soluble glutelins are included in the NIR protein estimates. Of these four groups, the albumins and globulins are most likely to end up in the steep fraction due to their chemical properties. In contrast, the alcohol-soluble prolamins and the alkaline-soluble glutelins are most likely to end up in the gluten fraction of the wet-milling procedure. A shift in relative concentrations of these different groups of proteins among a variety of genotypes may, therefore, result in shifts in rank when results from NIR protein and gluten concentration based on wet-milling analysis data are compared. This result could reduce the correlation between the two measurements.

The germ is typically the major source of oil. However the contribution of the germ in the wet-milling procedure is much lower than was expected. The germ recovery is smaller than estimated oil concentration in the kernel obtained using NIR (Table I). The possibility exists that the germs in this material are too brittle to be recovered effectively in the germ-fiber extraction steps. If this is the case, some of the germ and more importantly, some of the oil, will eventually end up in the fiber fraction and therefore contribute to overestimation of fiber.

Two fractions isolated by the wet-milling procedure have no counterparts in the NIR analysis. The steepwater solids fraction consists of all soluble components and includes minerals, vitamins, soluble protein, and soluble sugars. The steepwater solids fraction of IHP is almost twice as large as that of ILP (Table I) suggesting the presence of higher amounts of soluble proteins. Furthermore, significant variation existed for steepwater solids concentration among the 200 $S_1$ families (Table II).

The largest fraction not accounted for using NIR is fiber. As with all other traits, the parental strains are very different in percentage of fiber (Table I). The IHP has ≈70% more fiber than the ILP. Wet-milling fiber is mechanically separated from other kernel components and may contain a large amount of attached starch and protein. The cleanliness and amount of the fiber fraction depends on the ease of component separation as well as fiber concentration. Thus, fiber values may be different than those which would be found by chemical analysis for fiber.

Repeatability values were very high for all traits measured using either of the two methods (Table II). With the exception of gluten concentration, repeatability estimates were as high or higher for wet-milling data as they were for NIR.

### Correlations

Starch extracted by wet-milling analysis was significantly positively correlated with NIR starch ($r_p$ = 0.82, $r_g$ = 0.89) and negatively correlated with NIR protein ($r_p$ = –0.83, $r_g$ = –0.89) (Table III). Gluten concentration was significantly positively correlated with NIR protein ($r_p$ = 0.72, $r_g$ = 0.80) and significantly negatively correlated with both wet-milling starch ($r_p$ = –0.80, $r_g$ = –0.82) and NIR starch ($r_p$ = –0.73, $r_g$ = 0.80). Fiber concentration was also highly negatively correlated with wet-milling starch ($r_p$ = –0.81, $r_g$ = –0.83). Lower but highly significant correlations ($P < 0.001$) were observed between the steepwater solids fraction and starch, protein, fiber, and germ concentrations. Germ concentration was significantly correlated ($P < 0.01$) with all traits except gluten and fiber concentration.

### QTL Analysis

Identification of molecular marker probes associated with a trait allows determination of genetic control of the trait being studied,

**TABLE III**
**Genotypic ($r_g$ [above diagonal]) and Phenotypic ($r_p$ [below diagonal]) Correlations Among Kernel Traits Measured Using Wet-Milling Analysis and Near-Infrared Reflectance (NIR) on a Population of $F_2S_1$ Families from a Cross of Illinois High Protein and Illinois Low Protein Corn (IHP × ILP)**

| | 100-g Wet-Milling Analysis | | | | | NIR | | |
|---|---|---|---|---|---|---|---|---|
| Trait | Starch | Gluten | Fiber | Germ | Steepwater Solids | Starch | Protein | Oil |
| Wet-milling | | | | | | | | |
| Starch | | –0.82 | –0.83 | –0.43 | –0.54 | 0.89 | –0.89 | –0.01 |
| Gluten | –0.80***[a] | | 0.57 | 0.06 | 0.20 | –0.81 | 0.80 | –0.21 |
| Fiber | –0.81*** | 0.52*** | | 0.08 | 0.39 | –0.77 | 0.76 | 0.05 |
| Germ | –0.44*** | 0.05 | 0.10 | | 0.49 | –0.23 | 0.23 | 0.21 |
| Steepwater solids | –0.50*** | 0.18* | 0.37*** | 0.43*** | | –0.45 | 0.44 | 0.23 |
| NIR | | | | | | | | |
| Starch | 0.82*** | –0.73*** | –0.70*** | –0.21** | –0.40*** | | –0.96 | 0.04 |
| Protein | –0.83*** | 0.72*** | 0.70*** | 0.23*** | 0.39*** | –0.92*** | | –0.11 |
| Oil | –0.05 | –0.18* | 0.08 | 0.23*** | 0.26*** | 0.01 | –0.10 | |

[a] ***, **, * = significant at 0.001, 0.01, and 0.05 probability levels, respectively.

provides information useful for manipulating the trait genetically, and may ultimately allow cloning of genes controlling the trait. Because large numbers of samples are needed to measure marker-trait associations, knowing the relationship of NIR determinations to wet-milling fractions is important.

Only probes showing an association with at least one trait at $P < 0.01$ are presented (Table IV). In general, wet-milling and NIR identified the same significant marker-QTL associations for starch. Exceptions were found on chromosomes 1 (Bnl8.052) and 5 (Umc72) where a highly significant association was found for wet-milling starch but not for NIR starch. Marker-associated effects for wet-milling starch were generally larger than for NIR starch. Significant marker associations for gluten were found in the same chromosomal regions as protein. However, the significance levels associated with gluten tended to be lower. Signs of marker effects for protein and gluten were opposite those for either wet-milling or NIR starch. Significant associations with fiber, a trait we have not studied previously, were similar to those for starch concentration. In all instances, when significant associations were found for both traits, the positive allele for starch was the negative allele for fiber concentration.

Markers with significant associations with extractable germ usually had significant associations with starch and protein concentration as well (Table IV). In nearly all instances, the marker allele positively associated with germ concentration was negatively associated with starch and positively associated with protein. Exceptions were Npi 328 on chromosome 3 and Umc104 and Umc108 on chromosome 5. Fewer significant associations were identified for steepwater solids concentration than for starch, protein, or gluten. However, markers with significant associations with steepwater solids nearly always had significant associations with starch. The allele associated with increased starch concentration was always associated with decreased steepwater solids concentration.

Fourteen markers had significant associations with germ concentration (Table IV). Of these, 13 also were significantly associated with either NIR or wet-milling starch with effects having opposite signs. Only four of these markers showed significant associations with oil. This result is surprising because most of the oil is stored in the germ, and germ size has been associated with oil concentration (Dudley et al 1972). However, the range in oil concentration in this cross was limited, resulting in a small number of markers showing significant associations with oil. In addition, the correlation between oil and germ is low (Table III). Thus, the lack of correspondence between germ and oil concentrations may have been due to the variation in oil concentration resulting from variation in concentration of oil in the germ or a confounding of germ size with germ brittleness. A more brittle germ will result in reduced recovery and, thus, lower estimates of germ concentration. Despite limited agreement between germ and oil, the marker with the largest effect for oil concentration (Npi238 on chromosome 3) also had the largest effect for germ concentration (Table IV).

## CONCLUSIONS

The high correlation between wet-milling starch and NIR starch suggests that NIR is effective in ranking progenies for extractable starch. In this population, selection for higher starch or lower

**TABLE IV**
**Marker Regression (*b* value, %) and Level of Significance for Traits Measured Using 100-g Wet-Milling Analysis and Near-Infrared Reflectance (NIR)**

| | | 100-g Wet-Milling Analysis | | | | | NIR | | |
|---|---|---|---|---|---|---|---|---|---|
| Probe | Bin[a] | Starch | Gluten | Fiber | Germ | Steepwater Solids | Starch | Protein | Oil |
| Bnl8.05 | 1.01 | 0.9**[b] | –0.3 | –0.2 | –0.38*** | –0.06* | 0.3 | –0.3* | –0.03 |
| Php20689 | 1.02 | 1.0** | –0.3 | –0.5** | –0.02 | –0.06* | 0.6** | –0.3* | –0.03 |
| Npi262 | 1.04 | 2.1*** | –0.8*** | –0.8*** | –0.22 | –0.10*** | 1.2*** | –0.8*** | 0.01 |
| Php20872 | 1.05 | 1.8** | –0.6* | –0.7** | –0.47** | –0.02 | 0.8** | –0.6*** | 0.05 |
| Umc67 | 1.06 | 1.5*** | –0.5** | –0.4* | –0.45*** | –0.08** | 0.7*** | –0.5*** | 0.05* |
| Umc23 | 1.07 | 2.2*** | –0.7*** | –0.9*** | –0.32** | –0.12*** | 1.2*** | –0.8*** | 0.00 |
| Umc128 | 1.08 | 1.5** | –0.6* | –0.5* | –0.26 | –0.07* | 0.9*** | –0.7*** | 0.03 |
| Npi238 | 1.11 | 1.6*** | –0.8*** | –0.5*** | –0.02 | –0.02 | 0.9*** | –0.5*** | 0.01 |
| Umc6 | 2.03 | 1.6*** | –0.6** | –0.6*** | –0.31** | –0.06* | 0.7*** | –0.5*** | 0.04 |
| Npi337 | 2.07 | 1.2** | –0.7*** | –0.6** | 0.07 | 0.03 | 0.7*** | –0.4** | –0.02 |
| Umc5 | 2.07 | 1.2* | –0.4 | –0.6** | 0.01 | 0.03 | 0.7** | –0.5** | 0.06* |
| Npi298 | 2.08 | 0.9* | –0.3 | –0.4* | –0.04 | –0.09** | 0.3 | –0.2 | 0.03 |
| Umc121 | 3.01 | 1.1** | –0.4* | –0.4* | –0.15 | –0.06* | 0.3 | –0.3* | –0.01 |
| Php20042 | 3.03 | 1.4*** | –0.7*** | –0.5*** | –0.07 | –0.06* | 0.6*** | –0.4** | –0.02 |
| Umc154 | 3.04 | 1.7*** | –0.8*** | –0.7*** | –0.02 | –0.05 | 0.9*** | –0.5*** | –0.02 |
| Bnl5.37 | 3.06 | 1.1* | –0.5* | –0.2 | –0.29* | –0.11** | 0.7** | –0.5** | –0.05 |
| Npi328 | 3.06 | 0.2 | –0.3 | –0.6 | 0.65** | 0.02 | 0.1 | –0.1 | 0.13** |
| Php10080 | 3.09 | 0.9* | –0.2 | –0.4* | –0.19 | –0.01 | 0.3 | –0.3** | –0.01 |
| Php20726 | 3.09 | 0.7 | –0.3 | –0.5* | 0.12 | 0.04 | 0.6* | –0.4** | 0.01 |
| Sh2 | 3.09 | 1.2** | –0.4* | –0.5** | –0.16 | –0.03 | 0.5** | –0.4** | 0.01 |
| Umc63 | 3.09 | 1.3** | –0.3 | –0.7*** | –0.21 | –0.01 | 0.6** | –0.5*** | –0.01 |
| Umc72 | 5.02 | 1.1** | –0.2 | –0.4* | –0.40*** | –0.06* | 0.2 | –0.2 | –0.05 |
| Php20746 | 7.04 | 1.1** | –0.4* | –0.3 | –0.26* | –0.07** | 0.5* | –0.4** | 0.02 |
| Php20020 | 7.06 | 0.9* | –0.4* | –0.4* | –0.11 | 0.00 | 0.7*** | –0.5*** | 0.03 |
| Bnl9.11 | 8.02 | 1.3** | –0.5* | –0.5** | –0.19 | –0.06 | 0.7** | –0.4** | –0.02 |
| Bnl9.08 | 8.03 | 1.3** | –0.7** | –0.6** | 0.08 | 0.00 | 0.8*** | –0.6*** | 0.06* |
| Npi260 | 8.03 | 0.1 | –0.3 | –0.1 | 0.22 | 0.04 | 0.4* | –0.2 | 0.07*** |
| Umc2 | 8.05 | 1.3*** | –0.5** | –0.4* | –0.20 | –0.07* | 0.7*** | –0.6*** | 0.04* |
| Umc94 | 8.06 | 1.1** | –0.5** | –0.4* | –0.13 | –0.07* | 0.6*** | –0.4*** | 0.04 |
| Npi268 | 8.07 | 0.7 | –0.3 | –0.3* | 0.05 | –0.01 | 0.5** | –0.3* | 0.03 |
| Umc7 | 8.08 | 0.8* | –0.1 | –0.4* | –0.15 | –0.07** | 0.5** | –0.3* | 0.00 |
| Php20075 | 9.03 | 1.7*** | –0.4 | –0.8*** | –0.35* | –0.07* | 0.8*** | –0.5** | –0.08** |
| Bnl14.28 | 9.06 | 0.3 | –0.5** | 0.0 | 0.28* | 0.05 | 0.4* | –0.2 | 0.02 |
| Bnl10.17 | 10.00 | 1.3*** | –0.3 | –0.5** | –0.35** | –0.09** | 0.6** | –0.5*** | 0.04 |
| Npi578 | 10.03 | 1.1** | –0.4 | –0.3 | –0.33** | –0.05 | 0.5* | –0.3* | 0.00 |
| Umc130 | 10.03 | 1.1** | –0.6** | –0.2 | –0.21 | –0.05 | 0.6*** | –0.4*** | 0.05* |
| Bnl7.49 | 10.07 | 0.3 | 0.1 | –0.3 | –0.03 | –0.10** | 0.1 | 0.0 | –0.03 |

[a] Bin indicates map location according to the University of Missouri maize map.
[b] ***, **, and * = significant at 0.001, 0.01, and 0.05 probability levels, respectively.

protein concentration based on NIR data was expected to be equally effective in increasing extractable starch concentration. This material has a wide range of starch and protein concentrations. Whether the wet-milling starch vs. NIR starch correlations are as high in material with a smaller range has yet to be determined. However, considering the costs and labor requirements of NIR and wet-milling analysis, it appears NIR is the method of choice for initial selection. For comparison of advanced material such as hybrids near release to the market, wet-milling analysis may be needed to determine wet-milling potential.

Chromosome regions identified as being associated with NIR starch also identified those associated with wet-milling starch. Likewise, markers associated with NIR protein were also associated with gluten. Thus, NIR can be used to identify chromosome regions associated with these wet-milling fractions.

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