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The Forage and Grain of MON 87460, a Drought-Tolerant Corn Hybrid, Are Compositionally Equivalent to That of Conventional Corn

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MON 87460 contains a gene that expresses cold shock protein B (CSPB) from *Bacillus subtilis*. Expression of this gene confers a yield advantage when yield is limited by water availability. Compositional analyses of MON 87460 and a conventional corn variety with similar background genetics were conducted on forage and grain harvested from multiple replicated field sites across the United States during the 2006 growing season and across Chile during the 2006−2007 growing season. The U.S. field trials were conducted under typical agronomic practices, whereas the Chilean field trials incorporated a strip-plot design that included well-watered and water-limited treatments. Results demonstrated that levels of the components analyzed were comparable between MON 87460, the conventional control, and the commercially available corn hybrids.

KEYWORDS: MON 87460; cold shock protein B; conventional corn; forage; grain

INTRODUCTION

The reduction in crop yields imposed by limited water availability can have far-reaching implications. Agriculture currently accounts for 70% of the fresh water used by humans. This rate of water use can locally exceed regeneration rates, often relying on underground aquifers that are rapidly being depleted (*1*). In North America, it is estimated that 40% of annual crop losses are due to suboptimal water availability (*2*). Modern biotechnological approaches to enhancing drought tolerance in plants now include modifying the expression of (i) functional proteins associated with the synthesis of osmoprotectants, (ii) transcription factors, (iii) scavengers of reactive oxygen species, and (iv) molecular chaperone proteins (*3*,*4*). Expression of cold shock protein B (CSPB), a molecular chaperone derived from *Bacillus subtilis*, may provide a yield advantage when corn (*Zea mays* L.) is subject to water restriction (*5*). Mechanistically, CSPB appears to function as a polynucleotide chaperone that serves to facilitate adaptations to periods of stress (see refs *6* and *7* for reviews on CSPB). Monsanto Company has developed drought-tolerant corn MON 87460 that provides a yield benefit when yield is limited by water availability (*5*). MON 87460 was produced by stable insertion of the coding sequence of CSPB from *B. subtilis*.

Comparisons of the levels of key nutrient and antinutrients in crops containing biotechnology-derived traits with those of conventional varieties represent an important consideration in nutritional and safety assessments (*8*−*14*). In consultation with government agencies, the Organization for Economic Cooperation and Development (OECD) has promoted a list of well-defined metabolic constituents for assessment in compositional studies of new crops, including those for maize (*15*, *16*). The purpose of this study was, based on OECD guidelines, to compare the composition of MON 87460 to a conventional corn variety with similar background genetics grown under the same conditions. Compositional analyses were conducted on forage and grain harvested from six replicated field sites across the United States during the 2006 growing season and three replicated field sites across Chile during the 2006−2007 growing season. The U.S. field trials were conducted under normal agronomic practices, whereas the Chilean field trials incorporated a strip-plot design that facilitated irrigation management for optimal yield and irrigation management for reduced water replacement. Components assessed in forage samples included proximates (protein, fat, ash, and moisture), carbohydrates by calculation, acid detergent fiber (ADF), neutral detergent fiber (NDF), calcium, and phosphorus. Components assessed in grain samples included proximates (protein, fat, ash, and moisture), carbohydrates by calculation, ADF, NDF, total detergent fiber (TDF), total amino acid composition, fatty acid composition, minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), vitamins [vitamin B_1 (thiamine), vitamin B_2 (riboflavin), vitamin B_6 (pyridoxine), vitamin E, niacin, and folic acid], furfural, raffinose, phytic acid, *p*-coumaric acid, and ferulic acid. A range of commercially available conventional corn hybrids were also included as reference substances for each field trial to provide data for the

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development of a 99% tolerance interval for each component analyzed.

MATERIALS AND METHODS

Corn Samples for Compositional Analysis. MON 87460 was produced using a CSPB coding sequence derived from *B. subtilis*, which encodes a protein that confers a yield advantage when yield is limited by water availability. Seeds of test, control, and reference corn were planted in the spring of 2006 at six sites in the United States. Locations of the field sites were as follows: Benton County, Iowa; Greene County, Iowa; Stark County, Illinois; Parke County, Indiana; Pawnee County, Kansas; and York County, Nebraska. At each field site, the seed-starting substances were planted in a randomized complete block design with three blocks. Each block (replicate) consisted of five plots with one plot for each test, control, and reference substance. Production was managed according to normal agronomic field practices. A total of 18 different commercial conventional corn hybrids were grown in the field trials to serve as reference substances. Each different reference substance was grown at one of the six field sites. Seeds of test, control, and reference corn were planted in the winter of 2006 at three sites in major corn-producing regions of Chile. Locations of the field sites were as follows: Colina, Region Metropolitana; Calera de Tango, Region Metropolitana; and Lumbreras, Region Metropolitana. The experiment was arranged in a strip-plot design with three replicates per site, with irrigation treatment (well-watered or water-limited) as the whole-plot and substance type as the subplot. The whole-plot factor was arranged as a randomized complete block design. The subplot factor was randomly arranged within each replication but was consistent across the whole-plot factor. A total of 12 different commercial conventional corn hybrids were grown to serve as reference substances. Each different reference substance was grown at one of the different sites under both water treatments. The well-watered irrigation treatment was managed to provide optimal grain yield. The water-limited irrigation treatment was managed to impose a drought stress by withholding irrigation during the late vegetative through early grain fill growth stages (i.e., approximately V10 through R2). The use of a strip-plot design allowed comparisons of the test and control substances under two separate irrigation regimens. Comparisons of the test and control substances from the water-limited treatments are discussed here; comparisons of the test and control substances from the well-watered treatment are presented in the Supporting Information.

For both the U.S. and the Chilean field trials, the genetic purity of the corn plants was maintained by bagging the tassels and ear shoots at anthesis and self-pollinating each plant by hand. The forage was collected at the late dough/early dent stage, and the grain was collected at normal kernel maturity. After harvest, samples were shipped to Monsanto (St. Louis, MO), where they were ground to a fine powder in the presence of dry ice and maintained frozen until required for compositional analysis. The identity of forage and grain samples was based on sample handling records and Southern blot or polymerase chain reaction analyses of genomic DNA isolated from grain tissue.

Compositional Analyses. Components assessed are described in the main text and listed in **Tables 1**−**7**. All compositional analyses were performed at Covance Laboratories, Inc. (Madison, WI). Brief descriptions of the methods utilized for the analyses are described below.

Proximate Analysis. Protein levels were estimated by determining the total nitrogen content using the Kjeldahl method (*17*, *18*). The protein was calculated from the total nitrogen using the formula $N \times 6.25$. The fat content of the grain was estimated by the Soxhlet extraction method (*19*). The fat content of the forage was determined by fat acid hydrolysis, followed by extraction with ether and hexane (*20*, *21*).

The ash content was determined by ignition in an electric furnace and quantitation of the ash by gravimetric analysis (*22*). The moisture content was determined by the loss of weight upon drying in a vacuum oven at 100 °C to a constant weight (*23*, *24*).

Carbohydrate levels were estimated using the fresh weight-derived data and the following equation (*25*):

$$\%\ \text{carbohydrate} = 100\% - (\%\ \text{protein} + \%\ \text{fat} + \%\ \text{ash} + \%\ \text{moisture})$$

Fiber Analysis. The ADF was estimated by treating the samples with an acidic boiling detergent solution to dissolve the protein, carbohydrate, and ash. An acetone wash removed the fats and pigments. The lignocellulose fraction was collected and determined gravimetrically (*26*). The NDF was estimated by treating the samples with a neutral boiling detergent solution to dissolve the protein, enzymes, carbohydrate, and ash. An acetone wash removed the fats and pigments. Hemicellulose, cellulose, and lignin fractions were collected and determined gravimetrically (*26*, *27*). For TDF duplicate samples were treated with α-amylase and digested with enzymes to break down starch and protein. Ethanol was added to each sample to precipitate the soluble fiber. The samples were filtered, and the residue was rinsed with ethanol and acetone to remove starch and protein degradation products and moisture. The protein content was determined for one of the duplicates; the ash content was determined for the other. The total dietary fiber in the sample was calculated using the protein and ash values (*28*).

Minerals. To estimate the levels of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc, inductively coupled plasma emission spectrometry was used as described in the AOAC methods (*29*, *30*) and by Dahlquist and Knoll (*31*). The sample was dried, precharred, and ashed overnight at approximately 500 °C. The ashed sample was treated with hydrochloric acid, taken to dryness, and placed in a solution of 5% (v/v) hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown samples, measured by the inductively coupled plasma, with the emission of a standard solution.

Amino Acid Composition. Three procedures described in the literature (*32*) were used to estimate the values for 18 amino acids in corn grain. The procedure for tryptophan required a base hydrolysis with sodium hydroxide. The sulfur-containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct hydrolysis with hydrochloric acid. The individual amino acids were then quantitated using an automated amino acid analyzer.

Fatty Acid Composition. The lipid in the grain samples was extracted and saponified with 0.5 N sodium hydroxide in methanol. The saponified mixture was methylated with 14% boron trifluoride:methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation (*33*).

Vitamin E. The vitamin E amount in the grain was determined following saponification to break down any fat and release the vitamin as described by Cort et al. (*34*). The saponified mixture was extracted with ethyl ether and then quantitated directly by high-performance liquid chromatography (HPLC) on a silica gel column.

Riboflavin. The amount of riboflavin was measured in grain samples following hydrolysis with dilute acid as described in the literature (*35*). The quantity of riboflavin in the sample hydrolysates was determined by comparing the growth of *Lactobacillus casei* measured turbidimetrically with the growth response in the presence of varying amounts of a riboflavin standard.

Thiamine. Thiamine was extracted by autoclaving the grain samples in the presence of weak acid followed by phosphatase digestion to release any bound thiamine (*36*−*38*). Thiamine was purified from the resulting solution by ion exchange chromatography and then converted to thiochrome with potassium ferricyanide. The thiochrome was extracted into isobutyl alcohol, and the levels were quantitated fluorometrically.

Folic Acid. Folic acid was analyzed using a published procedure (*39*, *40*) in which the grain was hydrolyzed by autoclaving in the presence of ascorbic acid. To release folic acid, the hydrolyzed material was digested by incubation for 18 h with an enzyme preparation from chicken pancreas. The quantity of folic acid in the sample was determined by comparing the growth of *L. casei* measured turbidimetrically with the growth response in the presence of varying amounts of a folic acid standard.

Pyridoxine. The sample was hydrolyzed with dilute sulfuric acid in the autoclave, and the pH was adjusted to remove interferences. The amount of pyridoxine was determined by comparing the growth response of the sample, using the yeast *Saccharomyces carlsbergensis*, with the growth response of a pyridoxine standard. The response was measured turbidimetrically (*41*). Results were reported as pyridoxine hydrochloride.

Phytic Acid. Phytic acid was quantitated in grain following extraction using ultrasonication as described by Lehrfeld (*42*, *43*). Purification and concentration of the extract were conducted using a silica-based anion

Table 1. Proximate, Fiber, and Mineral Compositions of Forage from MON 87460 Corn

	U.S. 2006 typical agronomic practices			Chilean 2006−2007 water-limited				
component[a]	MON 87460 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	MON 87460 mean (range) [d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	literature (range)[d]	ILSI[h] (range)[d]
ash	3.76 (2.17−5.34)	4.21 (2.94−8.01)	(2.67−4.43) [1.52, 5.75]	5.29 (4.51−6.29)	5.49 (4.59−6.90)	(4.80−6.62) [3.59, 7.93]	2.43−9.64[f] (2−6.6)[g]	1.527−9.638
carbohydrates	86.45 (83.78−88.75)	85.77 (81.88−89.26)	(84.97−88.89) [82.09, 90.80]	85.92 (84.14−88.81)	86.02 (84.51−87.52)	(84.11−87.54) [81.74, 90.41]	76.5−87.3[f] (83.2−91.6)[g]	76.4−92.1
moisture	70.94 (64.70−77.90)	71.46 (66.50−75.70)	(64.20−75.50) [59.32, 81.14]	74.98 (72.00−77.40)	75.42 (73.00−77.60)	(73.40−77.50) [70.85, 80.94]	56.5−80.4[f] (55.3−75.3)[g]	49.1−81.3
protein	7.56 (6.65−8.57)	7.85 (6.45−10.24)	(5.80−8.63) [4.92, 10.30]	7.47 (5.49−8.76)	7.67 (6.52−9.14)	(5.56−8.59) [2.94, 11.20]	4.98−11.56[f]	3.14−11.57
fat	2.23 (1.07−3.24)	2.17 (1.28−2.88)	(1.60−3.62) [0, 4.67]	1.32[i] (0.50−1.92)[k]	0.84 (0.20−1.66)	(0.20−1.76) [0, 3.25]	1.42−4.57[f] (0.35−3.62)[g]	0.296−4.570
ADF	24.10 (17.78−34.43)	24.64 (19.11−29.21)	(19.44−30.49) [13.04, 35.77]	27.15 (23.03−32.00)	26.73 (23.10−30.79)	(20.73−33.39) [11.54, 42.87]	17.5−38.3[f] (18.3−41.0)[g]	16.13−47.39
NDF	38.69 (31.10−49.44)	38.75 (27.73−48.35)	(32.12−49.62) [24.23, 56.48]	39.06 (33.29−44.10)	40.10 (31.81−50.61)	(36.08−49.33) [25.58, 58.01]	27.9−54.8[f] (26.4−54.5)[g]	20.29−63.71
calcium	0.21 (0.14−0.30)	0.22 (0.13−0.33)	(0.12−0.25) [0.044, 0.35]	0.32 (0.20−0.44)	0.34 (0.26−0.41)	(0.21−0.37) [0.085, 0.50]	0.0969−0.3184[g]	0.0714−0.5768
phosphorus	0.18 (0.14−0.22)	0.19 (0.14−0.23)	(0.090−0.26) [0.074, 0.32]	0.16 (0.14−0.18)	0.17 (0.15−0.21)	(0.13−0.19) [0.077, 0.23]	0.1367−0.2914[g]	0.0936−0.3704

[a] Percent dry weight of sample, except moisture. [b] Nontransgenic control. [c] Commercial references planted at each site. [d] The range denotes the lowest and highest individual values across all sites. [e] The tolerance interval is specified to contain 99% of the commercial corn population where negative limits are set to zero. [f] Ridley et al. (*53*). [g] Sidhu et al. (*54*). [h] International Life Sciences crop composition database; Ridley et al. (*55*). [i] ADF, acid detergent fiber; NDF, neutral detergent fiber. [k] Statistically and significantly different ($p < 0.05$) from control.

Table 2. Proximate and Fiber Compositions of Grain from MON 87460 Corn

	U.S. 2006 typical agronomic practices			Chilean 2006−2007 water-limited				
component[a]	MON 87460 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	MON 87460 mean (range)[d]	control[b] mean (range) [d]	commercial references[c] (range)[d] [99% tolerance interval][e]	literature (range)[d]	ILSI[l] (range)[d]
ash	1.54[n] (1.33−1.83)[n]	1.46 (1.32−1.79)	(1.17−2.01) [0.55, 2.30]	1.47 (1.24- 1.75)	1.50 (1.39−1.63)	(1.27−1.63) [1.06, 1.93]	0.89−6.28[f] (1.1−3.9)[g]	0.616−6.282
carbohydrates	84.22 (81.40−87.04)	84.10 (81.31 −86.05)	(82.11−87.06) [80.32, 89.92]	84.21 (82.64−85.64)	84.10 (82.95−85.98)	(82.10−85.17) [80.40, 87.76]	77.4−87.2[f] (82.2−88.1)[h]	77.4−89.5
moisture	9.94 (9.12−11.00)	10.09 (9.17−11.20)	(8.74−11.30) [7.58, 12.13]	12.10 (11.60−12.50)	11.98 (11.30−12.50)	(11.70−13.20) [10.50, 14.11]	7−23[g] (8.18−26.2)[f]	6.1−40.5
protein	10.50 (8.19−13.21)	10.74 (8.77−13.33)	(8.27−11.50) [6.26, 13.45]	10.30 (9.41−11.45)	10.44 (9.17−11.50)	(9.99−12.19) [8.12, 13.56]	6−12[g] (9.7−16.1)[j]	6.15−17.26
fat	3.74 (3.44−4.06)	3.71 (3.57−3.96)	(2.95−4.40) [2.08, 5.12]	4.02 (3.71−4.28)	3.96 (3.47−4.23)	(3.18−4.22) [2.07, 5.10]	2.48−4.81[f] (3.1−5.7)[g]	1.742−5.823
ADF	3.03 (1.57−4.94)	3.02 (1.94−4.08)	(1.82−4.48) [0.62, 5.72]	2.59 (1.85−3.58)	2.33 (1.83−3.05)	(1.83−3.39) [0.88, 4.63]	2.46−11.34[f,h] (3.3−4.3)[g]	1.82−11.34
NDF	8.97 (6.45−11.63)	8.95 (7.82−12.22)	(6.51−12.28) [3.45, 15.08]	8.87 (7.33−11.31)	8.22 (7.91−8.66)	(6.08−10.36) [2.87, 13.22]	7.58−15.91[f] (8.3−11.9)[g]	5.59−22.64
TDF[m]	12.59 (10.42−14.57)	12.15 (10.76−14.87)	(10.65−16.26) [8.11, 17.95]	12.48 (10.78−14.43)	12.15 (11.06−13.70)	(10.57−14.56) [6.50, 17.54]	10.99−11.41[k]	8.82−35.31

[a] Percent dry weight of sample, except moisture. [b] Nontransgenic control. [c] Commercial references planted at each site. [d] The range denotes the lowest and highest individual values across all sites. [e] The tolerance interval is specified to contain 99% of the commercial corn population where negative limits are set to zero. [f] Sidhu et al. (*54*). [g] Watson (*56*). [h] Ridley et al. (*53*). [j] Jugenheimer (*57*). [k] Choi et al. (*58*). [l] International Life Sciences crop composition database; Ridley et al. (*55*). [m] TDF, total dietary fiber. [n] Statistically and significantly different ($p < 0.05$) from control.

Table 3. Mineral Composition of Grain from MON 87460 Corn

	U.S. 2006 typical agronomic practices			Chilean 2006−2007 water-limited				
component[a]	MON 87460 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range) [d] [99% tolerance interval][e]	MON 87460 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range) [d] [99% tolerance interval][e]	literature (range)[d]	ILSI[h] (range)[d]
calcium	0.0054 (0.0047−0.0061)	0.0054 (0.0048−0.0063)	(0.0036−0.0068) [0.0019, 0.0076]	0.0052 (0.0046−0.0065)	0.0050 (0.0041−0.0063)	(0.0035−0.0070) [0, 0.010]	0.01−0.1[f,g]	0.00127−0.02084
copper	1.89 (1.47−4.61)	1.86 (1.54−3.43)	(1.14−2.56) [0.39, 3.21]	2.19 (1.88−2.49)	2.12 (1.87−2.30)	(1.39−2.76) [0.22, 3.82]	0.9−10[f,g]	0.73−18.50
iron	18.24 (15.02−24.88)	18.30 (14.17−20.58)	(16.89−23.40) [13.28, 26.47]	17.67 (16.38−19.27)	18.60 (16.12−22.21)	(15.90−24.66) [7.05, 30.38]	1−10[f,g]	10.42−49.07
magnesium	0.11 (0.095−0.13)	0.12 (0.095−0.13)	(0.091−0.14) [0.059, 0.16]	0.13 (0.11−0.14)	0.13 (0.10−0.14)	(0.11−0.14) [0.083, 0.16]	0.09−1[f,g]	0.0594−0.194
manganese	6.79 (5.02−8.64)	6.89 (5.50−8.34)	(4.83−8.05) [2.27, 9.92]	6.71 (5.28−8.66)	6.54 (5.25−7.76)	(4.78−9.35) [0.72, 11.82]	0.7−54[f,g]	1.69−14.30
phosphorus	0.31 (0.27−0.35)	0.32 (0.27−0.37)	(0.24−0.36) [0.20, 0.40]	0.32 (0.25−0.36)	0.33 (0.27−0.38)	(0.30−0.38) [0.25, 0.42]	0.26−0.75[f,g]	0.147−0.533
potassium	0.38 (0.36−0.39)	0.38 (0.35−0.39)	(0.29−0.37) [0.26, 0.42]	0.40 (0.37−0.43)	0.40 (0.37−0.43)	(0.36−0.43) [0.29, 0.49]	0.32−0.72[f,g]	0.181−0.603
zinc	20.86 (18.24−24.75)	21.24 (17.41−25.20)	(16.78−28.17)[11.61, 32.63]	23.30 (18.36−26.77)	24.37 (21.29−27.79)	(18.25−30.44) [6.01, 42.60]	12−30[f,g]	6.5−37.2

[a] Percent dry weight of sample, except iron, manganese and zinc which are expressed as mg/kg dry weight. [b] Nontransgenic control. [c] Commercial references planted at each site. [d] K.ange denotes the lowest and highest individual values across all sites. [e] Tolerance interval is specified to contain 99% of the commercial corn population where negative limits are set to zero. [g] Watson (56)). [f] Watson ((59)). [h] International Life Sciences crop composition database, Ridley et al. ((55).

exchange column followed by quantitation using a polymer HPLC column (PRP-1, 5 mm, 150 mm × 4.1 mm) fitted with a refractive index detector.

Ferulic and p-Coumaric Acids. Ferulic and *p*-coumaric acids were assayed in grain using the method of Hagerman and Nicholson (*44*) in which the samples were extracted with methanol, and the extracts were hydrolyzed using 4 N sodium hydroxide, neutralized, and filtered. The levels of ferulic and *p*-coumaric acids were determined by reversed-phase HPLC with UV detection.

Furfural. The levels of furfural were determined using the method of Albala-Hurtado et al. (*45*), in which the corn grain was extracted with 4% trichloroacetic acid, centrifuged, filtered, concentrated, and analyzed by reversed-phase HPLC with UV detection. The limit of quantitation (LOQ) for furfural was 0.5 ppm based on fresh weight.

Raffinose. The raffinose assay was based on two methods (*46*, *47*) in which the grain samples were extracted with deionized water, and the extracts were treated with a solution of hydroxylamine hydrochloride in pyridine containing phenyl-α-D-glucoside as an internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoracetic acid and analyzed by gas chromatography with flame ionization detection.

Statistical Analysis of Composition Data. In all, 77 different analytical components were measured (9 in forage and 68 in grain). The following 15 analytes with > 50% of the observations at or below the LOQ of the assay were excluded from statistical analysis: sodium, furfural, 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 γ-linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, and 20:4 arachidonic acid. In the U.S. field trial, a total of 13 16:1 palmitoleic acid observations and one 22:0 behenic acid observation fell below the assay LOQ. To include a complete data set for 16:1 palmitoleic acid and 22:0 behenic acid, values equal to half the LOQ were assigned for the missing data points. A total of 62 components (9 forage and 53 grain) were therefore available for statistical evaluation of the US field trials. There were > 50% of 16:1 pamitoleic acid observations below the assay LOQ in the Chile trials and therefore there were a total of 61 components (9 forage and 52 grain) available for statistical evaluation of the Chilean field trials. Except for moisture and fatty acids, all component values were converted from a fresh weight to a dry weight basis and into their respective units.

PRESS residuals were used to identify outliers. A PRESS residual is the difference between any value and its predicted value from a statistical model that excludes the data point. The studentized version scales these residuals so that the values tend to have a standard normal distribution when outliers are absent. Thus, most values are expected to be between ±3. Extreme data points that were outside of the ±6 studentized PRESS residual range were considered for exclusion, as outliers, from the final analyses. One copper value for a reference substance from the Colina, Chile, site was excluded

Components were statistically analyzed using a mixed-model analysis of variance. The replicated sites were analyzed both individually (individual site data not presented) and in combined-site analyses. The combined-site analyses for the U.S. field trials used the model:

$$Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk}$$

where Y_{ijk} = unique individual observation, U = overall mean, T_i = substance effect, L_j = random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by substance interaction effect, and e_{ijk} = residual error. For each compositional component, the forage and harvested seed from the test substance were compared to the conventional control.

The combined-site analyses for the Chilean field trials used the model:

$$Y_{ijkl} = U + L_i + B(L)_{ij} + T_k + LT_{ik} + TB(L)_{ijk} + S_l + SB(L)_{ijl} + TS_{kl} + LS_{il} + LTS_{ikl} + e_{ijkl}$$

where Y_{ijkl} = unique individual observation, U = overall mean, L_i = random location effect, $B(L)_{ij}$ = random block within location effect, T_k = irrigation treatment effect, LT_{jk} = random location by treatment interaction effect, $TB(L)_{ijk}$ = random treatment by block within location interaction effect, S_l = substance effect, $SB(L)_{ijk}$ = random substance by

Table 4. Total Amino Acid Composition of Grain from MON 87460 Corn

	U.S. 2006 typical agronomic practices			Chilean 2006−2007 water-limited			
component[a]	MON 87460 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	MON 87460 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	ILSI[f] (range)[d]
alanine	0.80 (0.60−1.04)	0.82 (0.64−1.04)	(0.60−0.91) [0.43, 1.08]	0.78 (0.67−0.85)	0.79 (0.68−0.89)	(0.77−0.96) [0.59, 1.09]	0.439−1.393
arginine	0.45 (0.33−0.54)	0.44 (0.38−0.52)	(0.34−0.51) [0.24, 0.60]	0.43 (0.41−0.44)	0.42 (0.34−0.47)	(0.41−0.50) [0.32, 0.56]	0.119−0.639
aspartic acid	0.65 (0.52−0.79)	0.66 (0.54−0.78)	(0.52−0.72) [0.39, 0.84]	0.65 (0.59−0.71)	0.65 (0.59−0.73)	(0.63−0.76) [0.52, 0.88]	0.335−1.208
cystine	0.23 (0.19−0.27)	0.23 (0.20−0.26)	(0.19−0.24) [0.15, 0.27]	0.23 (0.22−0.25)	0.23 (0.20−0.24)	(0.20−0.26) [0.15, 0.30]	0.125−0.514
glutamic acid	2.07 (1.52−2.66)	2.09 (1.64−2.67)	(1.54−2.32) [1.06, 2.76]	2.01 (1.74−2.21)	2.03 (1.71−2.29)	(1.94−2.44) [1.51, 2.80]	0.965−3.536
glycine	0.39 (0.33−0.45)	0.39 (0.34−0.43)	(0.33−0.42) [0.26, 0.47]	0.36 (0.34−0.39)	0.36 (0.33−0.39)	(0.35−0.42) [0.30, 0.45]	0.184−0.539
histidine	0.32 (0.25−0.38)	0.32 (0.27−0.37)	(0.25−0.33) [0.20, 0.36]	0.31 (0.28−0.32)	0.31 (0.27−0.34)	(0.27−0.33) [0.23, 0.36]	0.137−0.434
isoleucine	0.38 (0.28−0.50)	0.38 (0.31−0.48)	(0.30−0.41) [0.22, 0.49]	0.37 (0.32−0.38)	0.37 (0.32−0.41)	(0.34−0.44) [0.27, 0.50]	0.179−0.692
leucine	1.41 (1.01−1.85)	1.43 (1.11−1.87)	(1.02−1.55) [0.68, 1.90]	1.36 (1.16−1.47)	1.37 (1.13−1.56)	(1.29−1.65) [0.98, 1.91]	0.642−2.492
lysine	0.30 (0.26−0.34)	0.30 (0.26−0.33)	(0.27−0.32) [0.22, 0.36]	0.29 (0.27−0.31)	0.29 (0.28−0.31)	(0.28−0.31) [0.25, 0.34]	0.172−0.668
methionine	0.22 (0.16−0.28)	0.22 (0.17−0.26)	(0.17−0.24) [0.14, 0.28]	0.20 (0.18−0.22)	0.20 (0.16−0.22)	(0.19−0.30) [0.095, 0.35]	0.124−0.468
phenylalanine	0.56 (0.41−0.72)	0.56 (0.45−0.72)	(0.43−0.61) [0.30, 0.74]	0.53 (0.46−0.58)	0.54 (0.45−0.61)	(0.51−0.63) [0.41, 0.72]	0.244−0.930
proline	1.01 (0.78−1.23)	1.02 (0.83−1.21)	(0.74−1.01) [0.56, 1.19]	0.96 (0.85−1.04)	0.97 (0.84−1.11)	(0.78−1.03) [0.64, 1.23]	0.462−1.632
serine	0.53 (0.40−0.64)	0.53 (0.43−0.67)	(0.39−0.60) [0.27, 0.70]	0.51 (0.45−0.58)	0.51 (0.43−0.59)	(0.48−0.60) [0.36, 0.71]	0.235−0.769
threonine	0.37 (0.30−0.45)	0.37 (0.31−0.45)	(0.29−0.40) [0.22, 0.46]	0.35 (0.32−0.39)	0.35 (0.31−0.39)	(0.33−0.39) [0.28, 0.44]	0.224−0.666
tryptophan	0.066 (0.054−0.088)	0.068 (0.055−0.085)	(0.047−0.070) [0.037, 0.081]	0.053 (0.046−0.059)	0.052 (0.042−0.063)	(0.047−0.070) [0.037, 0.081]	0.0271−0.215
tyrosine	0.32 (0.16−0.43)	0.30 (0.15−0.43)	(0.13−0.37) [0.0046, 0.54]	0.29 (0.18−0.33)	0.24 (0.12−0.35)	(0.25−0.41) [0.12, 0.52]	0.103−0.642
valine	0.52 (0.40−0.64)	0.52 (0.43−0.62)	(0.42−0.54) [0.33, 0.62]	0.50 (0.44−0.51)	0.51 (0.45−0.55)	(0.47−0.58) [0.39, 0.64]	0.266−0.855

[a] Percent dry weight of sample. [b] Nontransgenic control. [c] Commercial references planted at each site. [d] The range denotes the lowest and highest individual values across all sites. [e] The tolerance interval is specified to contain 99% of the commercial corn population where negative limits are set to zero. [f] International Life Sciences crop composition database; Ridley et al. (*55*). Most literature reports on amino acid composition express values as a percent of total amino acids. When expressed as percent dry weight, comparisons of study data with historical data are limited to results recorded in ILSI.

Table 5. Fatty Acid Content Composition of Grain from MON 87460 Corn

	U.S. 2006 typical agronomic practices			Chilean 2006−2007 water-limited				
component[a]	MON 87460 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	MON 87460 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	literature (range)[d]	ILSI[g] (range)[d]
16:0 palmitic	12.12 (11.60−15.21)	11.94 (11.45−12.38)	(8.80−13.33) [6.35, 16.03]	11.06 (10.54−11.33)	11.18 (10.75 − 11.45)	(9.84−12.33) [7.71, 14.14]	7−19[f]	7.94−20.71
16:1 palmitoleic	0.17 (0.15−0.20)	0.17 (0.15−0.23)	(0.059−0.15) [0, 0.21]				1[f]	0.095−0.447
18:0 stearic	2.05 .(1.88−2.34)[h]	1.98 (1.80−2.10)	(1.36−2.14) [1.00, 2.51]	1.86 (1.73−1.95)	1.86 (1.68−2.08)	(1.30−2.10) [0.71, 2.57]	1−3[f]	1.02−3.40
18:1 oleic	20.26−(19.32−21.08)	20.49 (19.50−21.77)	(21.17−33.71) [11.92, 39.78]	20.99 (20.20−21.60)	20.83 (19.59−21.98)	(20.78−29.13) [12.15, 35.55]	20−46[f]	17.4−40.2
18:2 linoleic	63.34 (59.90−65.07)	63.34 (61.88−64.70)	(49.31−62.94) [45.91, 72.47]	64.29 (63.27−65.10)	64.30 (62.75−65.65)	(56.51−64.46) [50.63, 72.71]	35−70[f]	36.2−66.5
18:3 linolenic	1.28 (1.17−1.46)	1.27 (1.22−1.33)	(0.89−1.56) [0.39, 1.85]	1.19 (1.13−1.25)	1.21 (1.12−1.26)	(1.03−1.38) [0.67, 1.76]	08−2[f]	0.57−2.25
20:0 arachidic	0.41 (0.39−0.44)	0.41 (0.37−0.45)	(0.30−0.49) [0.23, 0.56]	0.31 (0.30−0.34)	0.32 (0.30−0.33)	(0.30−0.41) [0.18, 0.52]	0.1−2[f]	0.279−0.965
20:1 eicosenoic	0.18 (0.17−0.19)[h]	0.19 (0.17−0.22)	(0.20−0.29) [0.15, 0.33]	0.18 (0.16−0.19)[h]	0.18 (0.17−0.20)	(0.18−0.27) [0.10, 0.34]		0.170−1.917
22:0 behenic	0.20 (0.14−0.27)	0.20 (0.14−0.27)	(0.069−0.28) [0, 0.37]	0.12 (0.058−0.20)	0.12 (0.059−0.15)	(0.062−0.18) [0, 0.32]		0.110−0.349

[a] Expressed as % of total fatty acid. The method included the analysis of the following fatty acids, which were not detected in the majority of samples analyzed; caprylic acid (8:0), capric acid (10:0), lauric acid (12:0), myristic acid (14:0), myristoleic acid (14:1), pentadecanoic acid (15.0), pentadecenoic acid (15:1), heptadecanoic acid (17:0), heptadecenoic acid (17:1), γ-linolenic acid (18:3), eicosadienoic acid (20:2), eicosatrienoic acid (20:3), and arachidonic acid (20:4). [b] Nontransgenic control. [c] Commercial references planted at each site. [d] The range denotes the lowest and highest individual values across all sites. [e] The tolerance interval is specified to contain 99% of the commercial corn population where negative limits are set to zero. [f] Watson (*59*). [g] International Life Sciences crop composition database; Ridley et al. (*55*). [h] Statistically and significantly different ($p < 0.05$) from control.

Table 6. Vitamin Composition of Grain from MON 87460 Corn

	U.S. 2006 typical agronomic practices			Chilean 2006−2007 water-limited				
component[a]	MON 87460 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	MON 87460 mean (range)[d]	control[b] mean (range)[d]	commercial references[c] (range)[d] [99% tolerance interval][e]	literature (range)[d]	ILSI[h] (range)[d]
folic acid	0.30 (0.25−0.36)	0.30 (0.24−0.35)	(0.19−0.31) [0.13, 0.38]	0.29 (0.25−0.37)	0.28 (0.23−0.35)	(0.26−0.42) [0.098, 0.58]	0.3[f]	0.147−1.464
niacin	18.59 (15.53−22.23)	18.52 (15.26−21.85)	(15.07−32.38) [4.67, 36.68]	18.54 (0.23−25.00)	21.73 (16.36−42.06)	(13.64−27.42) [2.23, 41.53]	9.3−70[f,g]	10.37−46.94
riboflavin/vitamin B_2	1.54 (0.95−2.04)	1.44 (0.94−1.94)	(0.95−2.42) [0.047, 2.91]	2.12 (1.43−2.89)	2.29 (1.64−2.81)	(1.81−2.78) [0.88, 3.61]	3−8.6[f,g]	0.50−2.36
thiamine HCl/vitamin B_1	3.31 (2.67−3.89)	3.21 (2.33−3.89)	(2.43−4.17) [1.84, 4.94]	3.10 (2.84−3.42)	2.98 (2.71−3.19)	(2.87−4.33) [1.55, 5.85]	0.25−5.6[f,g]	1.26−40.00
pyridoxine HCl/ vitamin B_6	6.10 (5.03−7.49)	6.24 (5.21−7.41)	(4.93−7.53) [3.12, 8.09]	6.17 (5.43−6.57)	6.15 (4.97−8.27)	(5.30−8.22) [2.06, 9.98]	5.3[f]; 9.6[g]	3.68−11.32
vitamin E	14.73 (11.09−20.02)	14.69 (9.47−18.44)	(5.96−17.70) [0, 26.07]	13.01 (12.16−14.24)	12.16 (10.15−13.64)	(2.84−15.53) [0, 22.61]	3−12.1[g] (17−47)[f]	1.5−68.7

[a] Expressed as mg/kg dry weight. [b] Nontransgenic control. [c] Commercial references planted at each site. [d] The range denotes the lowest and highest individual values across all sites. [e] The tolerance interval is specified to contain 99% of the commercial corn population where negative limits are set to zero. [f] Watson (*56*). [g] Watson (*59*). [h] International Life Sciences crop composition database; Ridley et al. (*55*).

Table 7. Antinutrient and Secondary Metabolite Composition of Grain from MON 87460 Corn

	U.S. 2006 typical agronomic practices			Chilean 2006−2007 water-limited				
component	MON 87460 mean (range)[c]	control[a] mean (range)[c]	commercial references[b](range)[c] [99% tolerance interval][d]	MON 87460 mean (range)[c]	control[a] mean (range)[c]	commercial references[a](range)[c] [99% tolerance interval][d]	literature (range)[c]	ILSI[i] (range)[c]
phytic acid (% DW)	0.83 (0.60−1.00)	0.84 (0.69−1.09)	(0.69−0.98) [0.50, 1.11]	0.79 (0.63−0.89)	0.77 (0.60−0.89)	(0.67−0.94) [0.40, 1.12]	0.48−1.12[e]	0.111−1.570
raffinose (% DW)	0.19 (0.15−0.22)	0.18 (0.15−0.22)	(0.079−0.19) [0.039, 0.26]	0.11 (0.087−0.14)	0.12 (0.097−0.15)	(0.061−0.15) [0, 0.21]	0.08−0.30[f]	0.020−0.320
ferulic acid (μg/g DW)	1772.22 (1561.63−1966.67)	1693.18 (1245.83−1997.77)	(1205.75−2873.05) [395.96, 3485.38]	1923.79 (1208.67−2352.27)	1852.11 (1088.34−2301.59)	(1011.40−2539.86) [0.4071.51]	113−1194[g] (3000)[h]	291.9−3885.8
p-coumaric acid (μg/g DW)	115.95 (99.45−136.67)	126.55 (94.77−156.25)	(128.21−327.39) [7.61, 408.53]	137.29 (85.52−168.18)	149.95 (66.48−208.43)	(84.15−259.68) [0, 378.67]	22−75[g]	53.4−576.2

[a] Nontransgenic control. [b] Commercial references planted at each site. [c] The range denotes the lowest and highest individual values across all sites. [d] The tolerance interval is specified to contain 99% of the commercial corn population where negative limits are set to zero. [e] Ridley et al. (53). [f] Watson (59). [g] Classen et al. (60). [h] Dowd and Vega (61). [i] International Life Sciences crop composition database; Ridley et al. (55).

block within location interaction effect, TS_{kl} = treatment by substance interaction effect, LS_{il} = random location by substance interaction effect, LTS_{ikl} = random location by treatment by substance interaction effect, and e_{ijkl} = residual error.

The reference substance data were used to develop population tolerance intervals for each field trial. A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured. For each compositional analyte, 99% tolerance intervals were calculated that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of conventional references. Each tolerance interval estimate was based upon one observation per unique reference substance. Because negative quantities are not possible, negative calculated lower tolerance bounds were set to zero.

SAS software was used to generate all summary statistics and perform all analyses (SAS Institute, 2002−2003). All statistical analyses were conducted by Certus International, Inc. (Chesterfield, MO).

RESULTS AND DISCUSSION

Overall, the results of the analyses of both forage and grain samples from the U.S. field trials showed that there were three significant differences ($p < 0.05$) between MON 87460 and the conventional control for three of the 62 comparisons from the combined-site analyses. These three significant differences included values for ash, 18:0 stearic acid, and 20:1 eicosenoic acid, all in grain. From the water-limited treatment of the Chilean field trials, there were significant differences ($p < 0.05$) between MON 87460 and the conventional control for two of 61 comparisons from the combined site analysis. These included values for forage fat and grain 20:1 eicosenoic acid. From the well-watered treatment of the Chilean field trials, the results of the analyses of both forage and grain samples showed that there were significant differences ($p < 0.05$) between MON 87460 and the conventional control for only two of 61 comparisons from the combined-site analysis. Further discussion of compositional data from the well-watered treatment is provided in the Supporting Information.

Proximate, Fiber, and Mineral Compositions. Compositional analysis results for forage and grain are presented in **Tables 1**−**3**. These results demonstrated that the levels of proximate components (moisture, fat, protein, and ash), carbohydrate by calculation, fiber (ADF, NDF, and TDF), and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, and zinc) in the grain as well as proximates, ADF, NDF, calcium, and phosphorus of forage from MON 87460 were comparable to those in the conventional control. From the U.S. field trials, no significant differences ($p > 0.05$) between MON 87460 and the conventional control were observed for these components with the exception of ash in grain (see **Table 2**). However, the relative magnitude of the difference, expressed as a percentage of the control value, was small (~5.6%).

No significant differences ($p > 0.05$) between MON 87460 and the conventional control were observed from the water-limited treatment of the Chilean field trial for proximate, fiber, and minerals, with the exception of fat in forage (see **Table 1**). The relative magnitude of the difference expressed as a percentage of the control value was ~57% with MON 87460 showing a value of 1.32% DW and the control showing a value of 0.84% DW. The corresponding values from the well-watered treatment (see the Supporting Information) were 1.16% DW for MON 87460 and 1.30% DW for the control, and no statistically significant difference ($p > 0.05$) was observed.

All proximate, fiber, and mineral values for MON 86470 were within the tolerance interval determined for commercial varieties evaluated for each respective field trial and within published ranges. These results demonstrated that proximate, fiber, and

mineral levels in the forage and grain of MON 87460 were within the same population as those of conventional, commercially available corn.

Total Amino Acid Composition. The levels of the 18 amino acids measured in the grain of MON 87460 were comparable to those in the grain of the conventional control (**Table 4**). No significant differences ($p > 0.05$) between MON 87460 and the conventional control were observed for these components (see **Table 4**) from either field trial. Furthermore, all amino acid values for MON 87460 were within the tolerance interval determined for commercial varieties evaluated for each respective field trial and within published ranges. These results demonstrated that the levels of these amino acids in the grain of MON 87460 were within the same population as those of conventional, commercially available corn.

Fatty Acid Composition. The levels of fatty acids in the grain of MON 87460 were comparable to those observed in the grain of the conventional control (**Table 5**). For the U.S. field trial, no significant differences ($p > 0.05$) between MON 87460 and the conventional control were observed for these components with the exceptions of 18:0 stearic acid and 20:1 eicosenoic acid. However, the relative magnitude of the differences expressed as a percentage of the control value was small (~3.5 and ~4.0%, respectively).

No significant differences ($p > 0.05$) between MON 87460 and the conventional control were observed with the exception of values for 20:1 eicosenoic acid in grain from the water-limited treatments of the Chilean field trial. The relative magnitude of the difference in 20:1 eicosenoic acid content, expressed as a percentage of the control value, was small (~4.4%).

All fatty acid values for MON 86470 were within the tolerance interval determined for commercial varieties evaluated for each respective field trial and within published ranges. These results demonstrated that the levels of these fatty acids in the grain of MON 87460 were within the same population as those of conventional, commercially available corn.

Vitamin Composition. Compositional analysis results showed that the levels of folic acid, niacin, riboflavin (vitamin B_2), thiamine (vitamin B_1), pyridoxine (vitamin B_6), and vitamin E in the grain of MON 87460 were comparable to those of the conventional control (**Table 6**). No significant differences ($p > 0.05$) between MON 87460 and the conventional control were observed for these components from either field trial. Furthermore, all vitamin values for MON 87460 were within the tolerance interval determined for commercial varieties evaluated at each respective field trial and within literature ranges. These results demonstrated that the levels of these vitamins in the grain of MON 87460 were within the same population as those of conventional, commercially available corn.

Antinutrient and Secondary Metabolite Composition. The metabolites phytic acid, raffinose, 2-furfural, ferulic acid, and *p*-coumaric acid have all been shown to be present in corn grain or processed corn components. Phytic acid is widely distributed in plants (*48*, *49*). Seeds accumulate up to 90% of stored organic phosphate as phytic acid, and it has been shown to limit the uptake of minerals such as calcium in higher animals. Raffinose is a nondigestible oligosaccharide that is considered to be an antinutrient due to gas production and the resulting flatulence caused by its consumption (*50*). Ferulic and *p*-coumaric acids in plants are derived from the aromatic amino acids, phenylalanine and tyrosine (*51*), and serve as precursors for a large group of phenylpropanoid compounds including flavonoids and coumarins. The nonstarch polysaccharide pentosans are a major source of furfural (*52*).

The levels of furfural were below the assay LOQ (< 0.5 ppm fresh weight) for all grain samples analyzed in this study. No significant differences ($p > 0.05$) between MON 87460 were observed for the antinutrient and secondary metabolites assessed here (**Table 7**) at either field trial. Furthermore, all values for MON 87460 were within the tolerance interval determined for commercial varieties evaluated in this study and within published ranges. These results demonstrated that the levels of these antinutrients and secondary metabolites in the grain of MON 87460 were within the same population as those of conventional, commercially available corn.

Conclusion. It has been shown that the genetic enhancement of conventional corn to produce the CspB protein did not produce significant changes in any of the key nutritional or antinutritional components analyzed in this study. It is particularly noteworthy that there were only five statistically significant differences ($p < 0.05$) from a total of 123 comparisons (or seven of 184 comparisons if data from the Chilean well-watered treatment are included; see the Supporting Information) and that for all but one of those, the relative magnitude differences, when expressed as difference from the control value, were less than 6%. The largest relative magnitude difference was that observed for fat values in forage (~57%) from the Chilean water-limited treatments, although much of this difference can be attributed to a greater effect of water limitation on control values. Overall, results of compositional analyses derived from two separate field trials demonstrated that the grain and forage of MON 87460 were comparable in levels of key nutrient and antinutrients to those of the conventional control and commercially available corn.

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Supporting Information Available: Results and discussion and tables for well-watered conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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