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Effect of the cp4-epsps Gene on Metal Bioavailability in Maize and Soybean Using Bionic Gastrointestinal Tracts and ICP-MS Determination

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ABSTRACT: The transformation and metabolism of dietary compounds are affected significantly by gut microbiota. Hence, gut microbiota are used to improve bionic gastrointestinal tracts. The effect of the cp4-epsps gene on metal bioavailability was proved by the comparison of the affinity-liposome metal content ratio (AMCR) in transgenic and conventional crops. The bioavailability of V, Mn, Co, Ga, Ag, Ba, and Pb in roundup ready soybean decreased significantly because the ratio of AMCR (R_{AMCR}) in the transgenic crop and its corresponding conventional type ranged from 0.36 to 0.69. In roundup ready maize, metal bioavailability decreased for Li and Cr (i.e., R_{AMCR} was 0.26 and 0.39, respectively) but increased for V, Co, and Pb (i.e., R_{AMCR} was 1.48, 2.07, and 2.12, respectively). Compared with conventional crops, safe dosage and maximum consumption of roundup ready crops were 1.59 times for soybean and 0.78 times for maize.

KEYWORDS: *bionic gastrointestinal tract, metal bioavailability, roundup ready crops, safe assessment, maize, soybean*

INTRODUCTION

The global area of transgenic crops has continued to increase impressively since 1996. In 2011, transgenic soybean and maize were principal transgenic crops, occupying 47% and 32% of total transgenic crops grown, respectively.[1] Agronomically important traits such as improving quality,[2,3] herbicide tolerance,[4−6] and insect resistance[7,8] have been introduced into soybean (*Glycine max*) and/or maize (*Zea mays*). Expression of the gene product (cp4-epsps) renders the tolerance of soybean and maize to glyphosate, which is the active ingredient in the roundup family of herbicides. To date, regulatory authorities in 12 countries have approved the environmental (commercial) release of at least one of the 30 plant lines expressing the protein cp4-epsps.[9]

However, the safety of transgenic crops has been a debated topic since the mid 1990s when the first genetically modified crop was released on the market.[10] The safe assessment of transgenic crops was focused on the variability of nutrition constituent,[11,12] the fate of transgenic plant DNA,[13] and Cry1AB protein[14] in the gastrointestinal tract. Metal compositions of transgenic crops are now frequently studied, but most of them were limited to the determination of total content.[15−17] To our best knowledge, the effect of gastrointestinal digestion on metal bioavailability in transgenic crops has not been reported. It is urgent to design valid methods for metal bioavailability assessment in transgenic crops.

Metal bioavailability in crops has been assessed by animal experiments[18] and in vitro digestion/Caco-2 cell model.[19,20] These methods are costly and rather complicated to perform.[21−23] Therefore, a biomimetic digestion and absorption system is important as a low-cost screening platform for rapidly identifying metal bioavailability in crops. The crops, such as soybean and maize, are rich in proteins and carbohydrates.[24,25] Gut microbiota not only provide additional enzymatic activities involved in the transformation of dietary compounds[26] but also has significantly enriched metabolism of glycans, amino acids, cholesterol, bile acids, and xenobiotics; methanogenesis; and 2-methyl-D-erythritol 4-phosphate pathway-mediated biosynthesis of vitamins and isoprenoids.[27,28] Metal ligands in the chyme could be affected by the digestion in stomach and intestine and gut microbiota metabolism. Therefore, gut microbiota should be inoculated into dynamic in vitro digestion models.[29] With the coexistence of gastrointestinal composition (including inorganics, organics, and digestive enzymes), intestinal microbiota are used to improve our previous biomimetic gastrointestinal digestive system.[30] As similar as the biomembrane between the gastrointestinal tract and blood vessels, the liposome was used as the gastrointestinal absorption model.[30] Affinity-liposome metals were used for metal bioavailability assessment as the bioassimilated part. On the basis of the biomimetic gastrointestinal digestive system and bionic biomembrane absorption model, a new bionic gastrointestinal tract was reconstituted. The effect of cp4-epsps on metal bioavailability in the crops was investigated by RAMCR, i.e., the metal bioavailability ratio between roundup ready crops and its corresponding conventional crops, at the same time, both safe dosage and maximum consumption values of roundup ready maize and soybean were analyzed.

MATERIALS AND METHODS

Apparatus. A RE-52 rotator evaporator (Ya Rong Biochemical Instrument Factory, China), a SHA-B temperature consistent oscillating water-bath (GuoHua Co., China), a MK-III microwave digestion system (Sineo Microwave Chemistry Technology Co., China), and an Agilent 7500cx series inductively coupled plasma

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Table 1. Total Metal Contents of Transgenic Soybean, Maize, and Their Counterparts (n = 3)

metals	roundup ready soybean (ng/g)	conventional soybean (ng/g)	roundup ready maize (ng/g)	conventional maize (ng/g)
V	23.4 ± 0.7	21.2 ± 0.5	33.9 ± 1.1	17.4 ± 0.7
Cr	172.6 ± 3.5	107.4 ± 3.2	174.6 ± 4.4	143.6 ± 4.3
Mn	31603.8 ± 821.7	28099.9 ± 786.8	13875.2 ± 416.3	6152.6 ± 190.7
Fe	181646.8 ± 9264.0	252608.1 ± 12377.8	161030.5 ± 7568.4	117245.6 ± 5268.3
Co	117.4 ± 4.7	214.5 ± 6.4	63.3 ± 2.8	64.1 ± 3.0
Cu	10238.7 ± 204.8	8315.9 ± 149.7	3959.4 ± 79.2	1603.5 ± 40.1
Zn	13711.2 ± 411.3	12421.8 ± 347.8	24129.2 ± 723.9	12595.5 ± 352.7
Cd	33.0 ± 1.0	35.9 ± 1.1	32.4 ± 1.0	25.3 ± 0.7
Pb	120.1 ± 3.8	111.8 ± 4.0	134.6 ± 4.0	119.1 ± 3.3

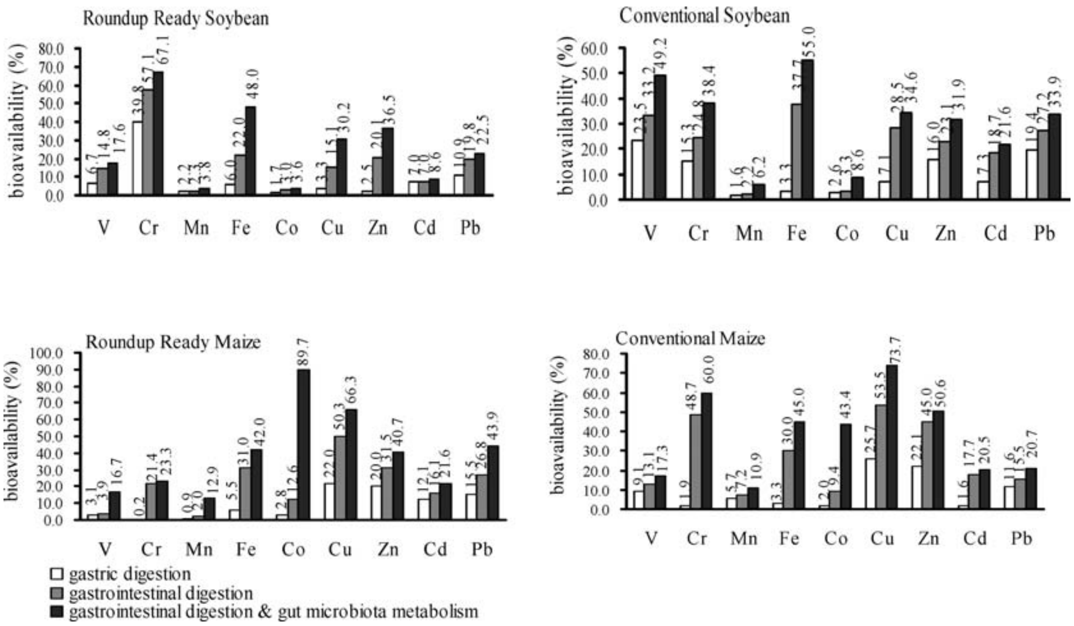

Figure 1. Comparison of metal bioavailability in four crops pretreated by three kinds of bionic digestion models (gastric digestion, gastrointestinal digestion, and gastrointestinal digestion and gut microbiota metabolism).

mass spectrometer (Agilent Technologies Co., USA) were used for metal determination. The pH values were measured using a Mettler Toledo 320-S pH meter (Mettler Toledo Co., China) with a combined electrode. Milli-Q purified water was obtained from a Milli-Q-purified water apparatus (Millipore Co., USA). Other equipment was used, including an 86C ULT ultra low temperature freezer (Thermo Electron Co., USA) and a ROTINA 420B high speed centrifuge (Hettich Co., Germany).

Chemicals. The biological chemicals such as α-amylase (1000 units/mg), pepsin (250 units/mg), lipase (200 units/mg), pancreatin (2000 units/mg), uric acid, mucin, bovine serum albumin, and bile were purchased from Sigma (St. Louis, MO, USA). Trypton, yeast extract, and glucose are supplied from Huangkai Microbial Sci. & Tech. Co. (Guangdong, China) and used for the preparation of microbiota tryptone−yeast extract−glucose (TYG) medium. Concentrated nitric acid, 69−70% (Merck KGaA, Germany), and hydrogen peroxide, 30% (Xilong Chemical CO., China), were used for the digestion of crop samples. All other chemicals were of analytical reagent grade from Shanghai Experiment Reagent Co., China, including lecithin, D-(+)-cellobiose, D-(+)-maltose, D-(−)-fructose, Tween 80, and meat extract.

The amounts of gastrointestinal inorganics, organics, and digestive enzymes, the process and digestion, and the digestion time were designed on human physiology.[31,32] Details of the components of saliva, gastric juice, duodenal juice, and bile fluid were described previously by us.[30] Milli-Q purified water (18.2 MΩ) was used for all sample preparations. To avoid metal contamination, all glassware and plastic ware were washed and kept for 48 h in 10% (v/v) nitric acid and then rinsed several times with ultrapure water before use.

Sample Preparation. Roundup ready soybean and maize containing cp4-epsps were provided by Zhangzhou Entry-Exit Inspection and Quarantine Bureau. Conventional soybean and maize were purchased from the Zhongmin supermarket in Zhangzhou, Fujian, China. The sample was washed by purified water three times and then dried at 80 °C to constant weight. The sample was ground in an agate mortar after cooling to room temperature.

Gut Microbiota Cultivation. Fecal samples were collected from three healthy volunteers. Samples were collected, on site, on the experimental day and were used immediately. Three samples were combined and then diluted 1:10 (w/v) with phosphate buffered saline (0.1 mol/L, pH 7.8) and homogenized for 2 min. According to the reference,[33] microbiota supplemented TYG medium was prepared. Then, 5 mL of supernate was diluted to 500 mL. Above this dilution (100 μL), they were inoculated to microbiota supplemented TYG medium at pH 7.8 and cultivated under anaerobic conditions, 37 °C for 48 h. Then, the intestinal microbiota were obtained and separated by centrifugation before used. Sterilization of microbiota nutrient solution, phosphate buffered saline, and instruments were done by autoclaving at 121 °C for 15 min.

Gastrointestinal Digestion, Gut Metabolism, and Absorption of the Crops in Bionic Gastrointestinal Tract. One gram of crop power was digested in a bionic mouth, stomach, and intestine at 37 °C on a gentle oscillation as follows. The bionic gastrointestinal digestion process was initiated with the addition of 5 mL of saliva and oscillated for 5 min to simulate chewing. Then, 30 mL of gastric juice was added to the above sample and incubated for 3 h. After gastric digestion, the chyme was adjusted to pH 7.8 ± 0.2 with 1 mol/L NaOH, mixed with 30 mL of duodenal juice and 15 mL of bile, and incubated on a gently rocking shaker for 7 h. During intestinal digestion, 15 mL of intestinal microbiota was also added into the chyme for simulating gut microbiota metabolism. Three kinds of sample pretreatment methods were investigated, including gastric digestion, gastrointestinal digestion, and gastrointestinal digestion and gut microbiota metabolism. Blank comparison was also obtained by using the same digestion procedure without the addition of the crops in each set of experiment. All chymes were filtered with a 0.45 μm membrane.

Egg-derived lecithin (0.1 g) was dissolved in chloroform and then transferred into a rotatory evaporator to evaporate chloroform. Twenty-five milliliters of chyme was mixed with liposome to form a homogeneous liposome suspension, frozen at −71 °C in a superlow freezer for 30 min, and then thawed at 37 °C. The freeze−thaw process was repeated 5 times to promote metal species distribution in the liposome−water system. Affinity-liposome metals could be separated from water-soluble metals by 0.22 μm membrane.

Determination of Metal Concentration in the Crop, Chyme, Affinity-Liposome Metal, and Water-Soluble Metal in the Chyme. Sample power (0.2 g) (soybean or maize) was weighed and transferred into a Teflon digestion vessel. The sample was added to 4.0 mL of concentrated HNO_3 and 2.0 mL of H_2O_2 (30%) and heated in a water bath at 80 °C until no smoke arose. The above sample or all of affinity-liposome metal was mixed with 2.0 mL of concentrated HNO_3 and 1.0 mL of H_2O_2 (30%) and decomposed under microwaves for 10 min under a pressure of 15 atm. After cooling to room temperature, the decomposed solution was diluted to 50 mL and used for metal determination by ICP-MS.

■ RESULTS AND DISCUSSIONS

Analysis of Total Metal Concentration in Soybean and Maize. Nine species of trace metals, including essential elements (V, Cr, Mn, Fe, Co, Cu, and Zn) and toxic elements (Cd and Pb) were found in transgenic and conventional crops, and the results are shown in Table 1. Four essential metals (Mn, Fe, Cu, and Zn) were rich at the level of microgram per gram in roundup ready soybean and maize. The content of other metals ranged from 17.4 ng/g to 214.5 ng/g. The content of most essential metals, including V, Cr, Mn, Cu, and Zn, in roundup ready soybean and maize was higher than that in conventional crops. The effect of gene cp4-epsps on metal concentration varied with the species of crops. Compared to conventional type crops, metal concentration in roundup ready crops was changed as follows. The concentration of metals (V, Mn, and Zn) was increased by 10%−12% for soybean but increased by 92%−126% for maize. Iron content was decreased by 28% for soybean, whereas it increased by 37% for maize. Chromium concentration increased to 172.6 ng/g from 107.4 ng/g for soybean and to 174.6 ng/g from 143.6 ng/g for maize. The concentration of toxic Cd and Pd varied under ±8% in soybean and increased by 13% and 28% in maize. After genetic modification, the unintended variation of metal accumulation or distribution in soybean and maize might be due to the difference of metal absorption capacity from soil .[34]

Effect of Gastrointestinal Digestion and Gut Metabolism on Metal Bioavailability. After bionic digestion, the product is referred to as the chyme. The metal species that could be released from crops, enter into the gastrointestinal tract and might be available for gastrointestinal absorption. The affinity-liposome metals in the chyme were metal complexes that could be absorbed by the gastrointestinal biomembrane. Metal bioavailability in the crop was assessed by the ratio of affinity-liposome metal content to total metal concentration.

Table 2. Metal Bioavailability in Transgenic Maize and Roundup Ready Soybean (n = 3)[a]

	roundup ready soybean			conventional soybean			roundup ready maize			conventional maize		
metal	AMC ng/g	bioavailability (%)	R_{AMCR}	AMC ng/g	bioavailability (%)	R_{AMCR}	AMC ng/g	bioavailability (%)	R_{AMCR}	AMC ng/g	bioavailability (%)	R_{AMCR}
V	4.1 ± 0.1	17.6 ± 0.6		10.4 ± 0.3	49.2 ± 1.4	0.36	5.7 ± 0.2	16.7 ± 0.5		2.0 ± 0.1	11.3 ± 0.4	1.48
Cr	53.2 ± 1.9	30.8 ± 1.1		41.2 ± 1.6	38.4 ± 1.5	0.80	40.7 ± 1.6	23.3 ± 0.9		86.2 ± 2.9	60.0 ± 2.0	0.39
Mn	1200.9 ± 50.4	3.8 ± 0.2		1742.2 ± 66.2	6.2 ± 0.2	0.61	1789.9 ± 68.0	12.9 ± 0.5		670.6 ± 26.8	10.9 ± 0.4	1.18
Fe	87190.5 ± 4533.9	48.0 ± 2.5		138934.5 ± 6391.0	55.0 ± 2.5	0.87	67632.8 ± 3381.6	42.0 ± 2.1		52760.5 ± 2849.1	45.0 ± 2.4	0.93
Co	4.2 ± 0.1	3.6 ± 0.1		18.4 ± 0.6	8.6 ± 0.3	0.42	56.8 ± 15.9	89.7 ± 25.1		27.8 ± 0.8	43.4 ± 1.2	2.07
Cu	3092.1 ± 77.3	30.2 ± 0.8		2877.3 ± 80.6	34.6 ± 1.0	0.87	2625.1 ± 78.8	66.3 ± 2.0		1181.8 ± 37.8	73.7 ± 2.4	0.90
Zn	5004.6 ± 180.2	36.5 ± 1.3		3962.6 ± 126.8	31.9 ± 1.0	1.14	9820.6 ± 294.6	40.7 ± 1.2		6373.3 ± 184.8	50.6 ± 1.5	0.80
Cd	6.1 ± 0.2	18.6 ± 0.7		7.8 ± 0.3	21.6 ± 0.9	0.86	7.0 ± 0.3	21.6 ± 0.9		5.2 ± 0.2	20.5 ± 0.8	1.05
Pb	27.0 ± 0.9	22.5 ± 0.7		37.9 ± 1.1	33.9 ± 0.9	0.66	59.1 ± 2.0	43.9 ± 1.5		24.7 ± 0.9	20.7 ± 0.7	2.12

[a] AMC = affinity-liposome metal content; bioavailability = the percent of AMC in total metal content in the crops. R_{AMCR} = ratio of metal bioavailability in roundup ready soybean and that in conventional type.

Table 3. Safe Dosage and Maximum Consumption of Roundup Ready Soybean and Maize^a

metal	RDAs/AIs values (mg/d)	UL/ML value (mg/d)	safe dosage of roundup ready soybean (g/d)	safe dosage of conventional soybean (g/d)	safe dosage of roundup ready maize (g/d)	safe dosage of conventional maize (g/d)	maximum consumption of roundup ready soybean (g/d)	maximum consumption of conventional soybean (g/d)	maximum consumption of roundup ready maize (g/d)	maximum consumption value of conventional maize (g/d)
V	ND	1.8					439024	173077	315789	900000
Cr	0.035, 0.025^b	0.06	658, 470^b	849.5, 606.8^b	860, 614^b	406.0, 290.0^b	1128	1456	1474	696
Mn	2.3, 1.8^b	11	1915, 1499^b	1320.2, 1033.2^b	1285, 1006^b	3429.8, 2684.2^b	9160	6314	6146	16403
Fe	8, 18^b	45	92, 206^b	57.6, 129.6^b	118, 266^b	151.6, 341.2^b	516	324	665	853
Co	ND	ND								
Cu	0.9	10	291	312.8	342	761.6	3234	3475	3809	8462
Zn	11, 8^b	40	2198, 1599^b	2776.0, 2018.9^b	1120, 815^b	1726.0, 1255.2^b	7993	10094	4073	6276
Cd		0.3					49180	38462	42857	57692
Pb		0.3					11111	7916	5076	12146

[a] Uls, tolerable upper intake levels; RDAs, recommended dietary allowances; AIs, adequate intakes; ND, not determinable due to the lack of data. [b] The data was suitable for females.

Roundup ready crops (soybean and maize) and their conventional types were pretreated by three kinds of bionic digestion models, i.e., gastric digestion, gastrointestinal digestion, and gastrointestinal digestion and gut microbiota metabolism. The influence of the digestion model on certain metal bioavailability in four kinds of crops was significant, and the result is shown in Figure 1. Only a fractional species of the metals could be absorbed by the bionic biomembrane. Bioenzymes and gut microbiota are part of the principal dynamics of gastrointestinal digestion and metabolism, and some metabolites are important metal ligands. After bionic digestion, the metals in the chyme from maize or soybean were transformed into their final coordinated metal complexes. According to the results of metal bioavailability in the chyme from four crops pretreated with different kinds of bionic digestion models, intestinal enzyme and gut microbiota could promote the digestion of metal ligands, and then metal species were transformed. Metal bioavailability was influenced by the bionic digestion model. For example, Co bioavailability in roundup ready and conventional maizes was increased obviously after gut microbiota metabolism as a result of the fact that the vitamin B contained in Co is derived from gut microbiota synthesis.[35] Because of the incorporation of gut microbiota into bionic gastrointestinal digestion, metal bioavailability could be increased in the range of 10%−610%. According to the above analysis, gastrointestinal digestion and gut microbiota metabolism could be a promising bionic gastrointestinal digestion model, and it could be combined with the liposome absorption model for the assessment of metal bioavailability.

Metal Bioavailability in Roundup Ready Soybean, Maize, and Their Corresponding Conventional Types. Metal bioavailability in roundup ready soybean, maize, and the comparison between transgenic and conventional types are described in Table 2. Low bioavailability for Co (3.6%) and Mn (3.8%), moderate bioavailability (17.6%−22.5%) for V, Cd, and Pb, and high bioavailability (30.2%−48.0%) for Cr, Fe, Cu, and Zn were found in roundup ready soybean. With respect to roundup ready maize, moderate bioavailability (12.9%−23.3%) for V, Cr, Mn, and Cd and high bioavailability (40.7%−89.7%) for other metals were found.

Compared to conventional crops, a significant difference of metal bioavailability in roundup ready crops was found. The difference was analyzed by R_{AMCR}, i.e., the ratio of metal bioavailability in roundup ready crops and that in corresponding conventional species. The bioavailability of all essential metals except for Zn and toxic metals (Cd and Pb) in roundup ready soybean was decreased. R_{AMCR} of V and Co was 0.36 and 0.42, respectively. R_{AMCR} of Mn and Pb was 0.61 and 0.66, respectively. R_{AMCR} of essential metals (Cr, Fe, and Cu) and toxic metal Cd ranged from 0.80 to 0.87. Only zinc bioavailability in roundup ready soybean was higher than that in conventional specie because its R_{AMCR} was 1.14.

Compared to conventional maize, the bioavailability of essential metals (Cr, Fe, Cu, and Zn) was decreased but increased for essential metals (V, Mn, and Co) and toxic metals (Cd and Pb) in roundup ready maize. R_{AMCR} of Cr was 0.39. R_{AMCR} of essential metals (Mn, Fe, Cu, and Zn) and toxic metal Cd ranged from 0.80 to 1.18. Bioavailability of essential metals (V and Co) and toxic metal Pb largely varied, and R_{AMCR} ranged from 1.48 to 2.12.

The content of phytic acid in roundup ready crops was higher than in conventional crops,[12] which could limit the bioavailability of metals such as iron, zinc, calcium, and selenium by the formation of indigestible chelates.[36,37] The content variation of L/D amino acids in transgenic crops has been reported,[11] and it could affect metal bioavailability because L-amino acids as metal ligands are generally susceptible to enzyme-catalyzed polymerization (translation) to structural and functional peptides and proteins.[38] The unexpected variation of quality traits in transgenic crops could be attributed to the expression process of genes, which might be interfered with by cp4-epsps. Metal bioavailability could be affected by the coexistence of other metals, for example, Fe contents in cereals is well correlated with the ratio of Fe/Zn.[39] The above factors may cause the difference in metal bioavailability between roundup ready crops and their conventional types.

Safe Dosage and Maximum Consumption of Roundup Ready Soybean and Maize. Deficiency of essential metals or metal overload is harmful for human health. Hence, safe dosage and maximum consumption of transgenic soybean and maize are important for the people who rely on cereal- and legume-based diets for their major sources of essential micronutrients. The effect of cp4-epspe on safe dosage and maximum consumption of transgenic soybean and maize should be evaluated. Safe dosage of roundup ready soybean or maize was calculated by the ratio of recommended dietary allowances (RDAs) or adequate intakes (AIs)[35,40] to affinity-liposome metal content (AMC). The maximum consumption value for transgenic soybean and maize was calculated by the ratio of tolerable upper intake levels (Uls)[35,40] to AMC. The results are shown in Table 3. The safe dosage of roundup ready soybean and maize for males was 92g/d and 118g/d, respectively. The safe dosage for females was 206g/d for roundup ready soybean and 266g/d for roundup ready maize. Maximum consumption of roundup ready soybean and maize for adults (including males and females) was 516g/d and 665g/d, respectively. Compared with conventional crops, safe dosage and maximum consumption of roundup ready crops were 1.59 times for soybean and 0.78 times for maize. Therefore, the effect of the cp4-epsps gene on metal nutrition and risk varied with the species of crops.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Global Status of Commercialized Biotech/GM Crops; ISAAA Brief 43, 2011. http://ations/briefs/43www.isaaa.org/resources/public/executivesummary/default.asp

(2) Dinkins, R. D.; Srinivasa, M. S. R.; Meurer, C. A.; Yan, B.; Trick, H.; Thibaud-nissen, F.; Finer, J. J.; Parrott, W. A.; Collins, G. B. Increased sulfur amino acids in soybean plants over expressing the maize 15 kDa zein protein. *In Vitro Cell. Dev. Biol. Plant* **2001**, *37*, 742−747.

(3) Drakakaki, G.; Marcel, S.; Glahn, R. P.; Lund, E. K.; Pariagh, S.; Fischer, R.; Christou, P.; Stoger, E. Endosperm-specific co-expression of recombinant soybean ferritin and aspergillus phytase in maize result in significant increases in the levels of bioavailable iron. *Plant Mol. Biol.* **2005**, *59*, 869−880.

(4) Green, J. M.; Owen, M. D. K. Herbicide-resistant crop: utilities and limitations for herbicide resistant weed management. *J. Agric. Food Chem.* **2011**, *59*, 5819−5829.

(5) Krishna, N. R.; Rimando, A. M.; Duke, S. O. Aminomethylphosphonic acid, a metabolite of glyphosate, causes injury in glyphosate-treated, glyphosate-resistant soybean. *J. Agric. Food Chem.* **2004**, *52*, 5139−5143.

(6) Pline, W. A.; Wu, J. R.; Hatzios, K. K. Effects of temperature and chemical additives on the response of transgenic herbicide-resistant soybeans to glufosinate and glyphosate applications. *Pestic. Biochem. physiol.* **1999**, *68*, 119−131.

(7) Meissle, M.; Lang, A. Comparing methods to evaluate the effects of Bt maize and insecticide on spider assemblags. *Agric. Ecosyst. Environ.* **2005**, *107*, 357−370.

(8) Gao, Y.; Schafer, B. W.; Collins, R. A.; Herman, R. A.; Xu, X. P.; Gilbert, J. R.; Ni, W. T.; Langer, V. L.; Tagliani, L. A. Characterization of Cry34AB1 and Cry35AB1 insecticidal crystal proteins expressed in transgenic corn plants and pseudomonas fluorescens. *J. Agric. Food Chem.* **2004**, *52*, 8057−8065.

(9) Center for Environmental Risk Assessment, ILSI Research Foundation.. A Review of the Environmental Safety of the CP4 EPSPS Protein. *Environ. Biosaf. Res.* **2011**, *10*, 5−25.

(10) Tabashnik, B. E. Seeking the root of insect resistance to transgenic plants. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 3488−3490.

(11) Herrero, M.; Ibáñez, E.; Martín-Álvarez, J. P. Alejandro, Cifuentes. Analysis of chiral amino acids in conventional and transgenic maize. *Anal. Chem.* **2007**, *79*, 5071−5077.

(12) Harrigan, G. G.; Ridley, P. W.; Riordan, G. S.; Nemeth, A. M.; Sorbet, R.; Trujillo, A. W.; Breeze, L. M.; Schneider, W. R. Chemical composition of glyphosate-tolerant soybean 40−3-2 grown in europe remains equivalent with that of conventional soybean (*Glycine max* L.). *J. Agric. Food Chem.* **2007**, *55*, 6160−6168.

(13) Netherwood, T.; Martín-Orúe, M. S.; O'Donnell, G. A.; Gockling, S.; Graham, J.; Mathers, C. J.; Gilber, J. H. Assessing the survival of transgenic plant DNA in the human gastrointestinal tract. *Nat. Biotechnol.* **2004**, *24*, 204−209.

(14) Lutz, B.; Wiedemann, S.; Einspanier, R.; Mayer, J.; Albrecht, C. Degradation of cry1ab protein from genetically modified maize in the bovine gastrointestinal tract. *J. Agric. Food Chem.* **2005**, *53*, 1453−1456.

(15) Li, X.; He, X. Y.; Luo, Y. B; Xiao, G. Y.; Jiang, X. B; Huang, K. L. Comparative analysis of nutritional composition between herbicide-tolerant rice with bar gene and its non-transgenic counterpart. *J. Food Compos. Anal.* **2008**, *21*, 535−539.

(16) Wang, Y.; Xu, W. T; Zhao, W. W; Hao, J. R; Luo, Y. B; Tang, X. G; Zhang, Y.; Huang, K. L. Comparative analysis of the proteomic and nutritional composition of transgenic rice seeds with Cry1ab/ac genes and their non-transgenic counterparts. *J. Cereal Sci.* **2012**, *55*, 226−233.

(17) Ridley, P. W.; Harrigan, G. G.; Breeze, L. M.; Nemeth, A. M.; Sidhu, S. R.; Glenn, C. K. Evaluation of compositional equivalence for multitrait biotechnology crops. *J. Agric. Food Chem.* **2011**, *59*, 5865−5876.

(18) Lonnerdal, B.; Mendoza, C.; Brown, H. K.; Rutger, N. J.; Raboy, V. Zinc absorption from low phytic acid genotypes of maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), and rice (*Oryza sativa* L.) assessed in a suckling rat pup model. *J. Agric. Food Chem.* **2011**, *59*, 4755−4762.

(19) Pixley, V. K.; Palacios-Rojas, N.; Glahn, P. R. The usefulness of iron bioavailability as a target trait for breeding maize (*Zea mays* L.) with enhanced nutritional value. *Field Crop. Res.* **2011**, *123*, 153−160.

(20) Aluru, R. M.; Rodermel, R. S.; Reddy, B. M. Genetic modification of low phytic acid 1−1 maize to enhance iron content and bioavailability. *J. Agric. Food Chem.* **2011**, *59*, 12954−12962.

(21) Hemalatha, S.; Gautam, S.; Platel, K.; Srinivasan, K. Influence of exogenous iron, calcium, protein and common salt on the bioaccessibility of zinc from cereals and legumes. *J. Trace Elem. Med. Biol.* **2009**, *23*, 75−83.

(22) Shen, L. H.; Luten, J.; Robberecht, H.; Bindels, J.; Deelstra, H. Modification of an in-vitro method for estimating the bioavailability of zinc and calcium from foods. *Z. Lebensm. Unters. Forsch.* **1994**, *199*, 442−445.

(23) Eisenbrand, G.; Pool-Zobel, B.; Baker, V.; Balls, M. B.; Blaauboer, B. J.; Boobis, A.; Carere, A.; Kevekordes, S.; Lhuguenot, J.-C.; Pieterse, R.; Kleiner, J. Methods of *in vitro* toxicology. *Food Chem. Toxicol.* **2002**, *40*, 193−236.

(24) Eldridge, C. A.; Black, T. L.; Wolf, J. W. Carbohydrate composition of soybean flours, protein concentrates, and isolates. *J. Agric. Food Chem.* **1979**, *27*, 799−802.

(25) Kasai, N.; Ikehara, H. Stepwise extraction of proteins and carbohydrates from soybean seed. *J. Agric. Food Chem.* **2005**, *53*, 4245−4252.

(26) Laparra, M. J.; Sanz, Y. Interactions of gut microbiota with functional food components and nutraceutials. *Pharmacol. Res.* **2010**, *61*, 219−225.

(27) Gill, R. S.; Pop, M.; Deboy, T. R.; Eckburg, B. P.; Turnbaugh, J. P.; Samuel, S. B.; Gordon, I. J.; Relman, A. D.; Fraser-Liggett, M. C.; Nelson, E. K. Metagenomic analysis of the human distal gut microbiome. *Science* **2006**, *312*, 1355−1359.

(28) Gosalbes, M. J.; Durbán, A.; Pignatelli, M.; Abellan, J. J.; Jiménez-Hernández, N.; Pérez-Cobas1, E. A.; Latorre, A.; Moya, A. Metatranscriptomic approach to analyze the functional human gut microbiota. *PLoS One* **2011**, *6*, e17447.

(29) Rajilić-Stojanović, M.; Maathuis, A.; Heilig, H. G. H. J.; Venema, K.; Vos, W. M.; Smidt, H. Evaluating the microbial diversity of an in vitro model of the human large intestine by phylogenetic microarray analysis. *Microbiology* **2010**, *156*, 3270−81.

(30) Li, S. X.; Lin, L. X.; Zheng, F. Y.; Wang, Q. X. Metal bioavailability and risk assessment from edible brown alga laminaria japonica, using biomimetic digestion and absorption system and determination by ICP-MS. *J. Agric. Food Chem.* **2011**, *59*, 822−828.

(31) Versantvoort, C. H. M.; Oomen, E.; Van de Kamp, E.; Rompelberg, C. J. M.; Sips, A. J. Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food Chem. Toxicol.* **2005**, *43*, 31−40.

(32) Oomen, A. G.; Rompelberg, C. J. M.; Bruil, M. A.; Dobbe, C. J. G.; Pereboom, D. P. K. H.; Sips, A. J. A. M. Development of an in vitro digestion model for estimation of bioaccessibility of soil contaminants. *Arch. Environ. Contam. Toxicol.* **2003**, *44*, 281−287.

(33) Goodman, L. A.; McNulty, P. N.; Zhao, Y.; Leip, D.; Mitra, D. R.; Lozupone, A. C.; Knight, R.; Gordon, I. J. Identifying genetic determinants needed to establish a human gut symbiont in its habitat. *Cell Host Microbe* **2009**, *6*, 279−289.

(34) Marengo, E.; Aceto, M. Statistical investigation of the differences in the distribution of metals in Nebbiolo-based wines. *Food Chem.* **2003**, *81*, 621−630.

(35) Subcommittee on the Tenth Edition of the Recommended Dietary Allowances, Food and Nutrition Board, Commission on Life Sciences, National Research Council. *Recommended Dietary Allowances*, 10th ed.; National Academy Press: Washington, D.C., 1989.

(36) Aluru, R. M.; Rodermel, R. S.; Reddy, B. M. Genetic modification of low phytic acid 1−1 maize to enhance iron content and bioavailability. *J. Agric. Food Chem.* **2011**, *59*, 12954−12962.

(37) Saha, P. R.; Weaver, C. M.; Mason, A. C. Mineral bioavailability in rats from intrinsically labeled whole wheat flour of various phytate levels. *J. Agric. Food Chem.* **1994**, *42*, 2531−2535.

(38) Friedman, M. Chemistry, nutrition, and microbiology of D-amino acids. *J. Agric. Food Chem.* **1999**, *47*, 3457−3479.

(39) Singh, V.; Garg, A. N. Availability of essential trace elements in Indian cereals, vegetables and spices using INAA and the contribution of spices to daily dietary intake. *Food Chem.* **2006**, *94*, 81−89.

(40) Trumbo, P.; Yates, A. A.; Schlicker, S.; Poos, M. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. *J. Am. Diet. Assoc.* **2001**, *101*, 294−301.