JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Comparison of Nutritional Traits Variability in Selected Eighty-Seven Inbreds from Chinese Maize (*Zea mays* L.) Germplasm

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Among cereals, only maize has not only a high amount of carotenoids, tocopherols, and oil content but also is rich in starch and protein content compared with other major food crops, such as rice and wheat. The present investigation was made primarily to assess the genetic variability for nutritionally important traits in 87 elite maize inbreds representing major heterotic groups in China. Carotenoid and tocopherol fractions were measured by high-performance liquid chromatography (HPLC), whereas oil, starch, and protein contents were detected by a VECTER22/N near-infrared analyzer. Significant interactions between genotypes and years were observed for all the traits. The pooled mean values of β-carotene, β-cryptoxanthin, α-carotene, lutein, zeaxanthin, and total carotenoids were 0.449, 0.876, 0.121, 5.803, 3.048, and 10.298 μg g^{-1}, respectively, whereas the combined mean performance of α-tocopherol, γ-tocopherol, δ-tocopherol, and total tocopherols were 23.98, 32.90, 2.189, and 59.55 μg g^{-1}, respectively. The average protein, starch, and oil contents were observed to be 12.28, 64.51, and 3.55%, respectively. High level of heritability estimates were observed for all the traits and ranged from 65.6% (protein content) to 92.5% (α/γ-tocopherol ratio). Most of the traits studied in this experiment were either significantly positive correlated or independent. The present finding exhibits substantial opportunities to the breeders for improvement of these traits in maize cultivars and also suggests further exploration of a new source of elite breeding stocks containing a high level of these nutritionally important compounds. Finally, these findings may also help in biofortification of maize.

KEYWORDS: ***Zea mays*; variability; nutritional traits; correlation; seed quality**

INTRODUCTION

The association of numerous physiological abnormalities with the intake of an imbalanced diet and malnutrition is a major health challenge in developed as well as developing countries. Development of high nutritional value staple food crops such as biofortified cereals may help in combating with these nutritional deficiencies like VAD (vitamin A deficiency). Humans and animals are incapable of carotenoid and tocopherol biosynthesis. Therefore, they depend on dietary carotenoid and tocopherol sources. In humans, the most prominent function of carotenoids and tocopherols is as vitamin A and E, respectively. The increase in bioavailability of carotenoids especially β-carotene has been reported with high amount of protein and fat, a component of oil (*1*). Besides higher biological activity of natural α-tocopherol over synthetic form of α-tocopherol, the consumer has a preference toward natural ingredients and has concerns about the toxic effects of synthetic carotenoids and tocopherols; high oil corn with enhanced amount of carotenoids and tocopherols may provide an alternative source of carotenoid and tocopherol supplements (*2*).

Among cereals, maize not only has a sufficient amount of carotenoids, tocopherols, and oil but also has a -comparable amount of starch and protein content compared with other major food crops such as rice and wheat. Among food crops, maize is the only principal source of non-provitamin A carotenoids especially lutein and zeaxanthin. Zeaxanthin is a rare carotenoid in nature and together with lutein is an essential component of the macular pigment in the eye (*3*). Starch and carotenoids (i.e., provitamin A (α-carotene, β-carotene, β-cryptoxanthin) as well as non-provitamin A carotenoids (lutein and zeaxanthin)) are primarily located in endosperm of maize grain (*4, 5*) whereas oil and tocopherols, consisting of mainly α-tocopherol, δ-tocopherol, and γ-tocopherol, are located in embryo (*6*). Protein found in both the endosperm and embryo occupies about 85% of the grain on a dry weight basis (*5*).

In 2002, a national survey on the status of nutrition and health of Chinese people showed that malnutrition especially VAD was more prevalent (*7*). In contrast to a dietary source of animal carotenoids and tocopherols, plant based carotenoids and

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10.1021/jf7037967 CCC: $40.75
Published on Web 07/12/2008

Table 1. Analysis of Variance (ANOVA), Mean (Standard Error), and Range for Nutritional Traits in Grain of 87 Maize Inbreds Grown during Summers 2004 and 2005

		joint mean square								
		g	y	y[a]g	error	mean (SE)			range	
traits	df	86	1	172	172	2004	2005	pooled	min	max
β-carotene (μg g^{-1})		0.424[b]	2.155[b]	0.025[b]	0.001	0.371 ± 0.003	0.528 ± 0.004	0.449 ± 0.003	0.016	1.726
β-cryptoxanthin (μg g^{-1})		1.851[b]	12.97[b]	0.143[b]	0.004	0.683 ± 0.006	1.069 ± 0.009	0.876 ± 0.007	0.012	3.666
α-carotene (μg g^{-1})		0.086[b]	0.167[b]	0.005[b]	0.0003	0.099 ± 0.001	0.143 ± 0.002	0.121 ± 0.002	0.004	0.852
lutein (μg g^{-1})		39.23[b]	234.5[b]	1.648[b]	0.123	4.983 ± 0.305	6.624 ± 0.377	5.803 ± 0.336	0.040	17.504
zeaxanthin (μg g^{-1})		8.606[b]	75.19[b]	0.457[b]	0.075	2.583 ± 0.143	3.513 ± 0.178	3.048 ± 0.157	0.022	6.728
total carotenoids (μg g^{-1})		66.94[b]	867.9[b]	4.007[b]	0.334	8.719 ± 0.391	11.877 ± 0.504	10.298 ± 0.439	0.096	22.495
α-tocopherol (μg g^{-1})		672.1[b]	2916.91[b]	19.90[b]	3.306	21.08 ± 1.311	26.89 ± 1.500	23.98 ± 1.389	3.980	74.81
γ-tocopherol (μg g^{-1})		1195.7[b]	620.2*	51.38[b]	3.881	31.95 ± 1.810	33.85 ± 2.024	32.90 ± 1.878	2.805	78.16
δ-tocopherol (μg g^{-1})		6.069[b]	0.267	0.708[b]	0.120	2.162 ± 0.125	2.217 ± 0.152	2.189 ± 0.132	0.236	5.338
total tocopherol (μg g^{-1})		1691.1[b]	6308.0[b]	86.32[b]	7.945	55.29 ± 2.186	63.81 ± 2.332	59.55 ± 2.204	19.60	120.69
α/γ tocopherol ratio		3.048[b]	2.856[b]	0.081[b]	0.006	0.957 ± 0.009	1.139 ± 0.010	1.048 ± 0.009	0.085	3.846
protein (%)		4.832[b]	55.94[b]	0.449[b]	0.062	11.88 ± 0.122	12.68 ± 0.124	12.28 ± 0.118	9.584	14.757
starch (%)		24.287[b]	74.06[b]	3.213[b]	0.657	64.97 ± 0.272	64.05 ± 0.290	64.51 ± 0.264	57.96	70.26
oil (%)		2.105[b]	1.388*	0.116[b]	0.024	3.613 ± 0.008	3.487 ± 0.008	3.550 ± 0.008	2.057	5.195
100-seed (g)		72.524[b]	0.276	5.293[b]	1.142	25.75 ± 0.460	25.81 ± 0.485	25.78 ± 0.457	17.44	37.66

[a] *P* at 0.001 significant. [b] *P* at 0.005 significant. df, degrees of freedom; g, genotype; y, year; SE, standard error.

tocopherols are more easily accepted among diverse socio-religious consumers and are in the reach of low socio-economic populations especially in rural areas of developing countries. In long-term, breeding for biofortication of major staple food crops such as maize with enhanced levels of carotenoids, tocopherols, oil, starch, and protein is not only a feasible way to cope with malnutrition but also may be more beneficial than synthetic carotenoids and tocopherols. However, without implementation of expensive strategies for the biofortification of major staple food crops by genetic engineering, it is more economical and free from public concerns to explore and utilize the genetic variations in the available germplasm of major food crops. However, there are few reports to evaluate nutritionally important traits systematically in maize. Thus, the aims of the investigation reported here were to assess variability for nutritionally important traits and their inter-relationships among elite maize germplasm representing major heterotic groups utilized in China (*8*).

MATERIALS AND METHODS

Plant Materials. In the present investigation, 87 elite inbreds representing major heterotic groups and extensively utilized in the Chinese maize breeding program for the production of commercial cultivars were selected and grown on an agronomy farm at China Agricultural University, Beijing, during summers 2004 and 2005 (see Supporting Information) (*8*). Each genotype was planted in a row of 4 m length apart 0.67 m row to row, with two repeats in complete randomized block design. Recommended commercial practices were followed to raise a good crop. For trait measurement, seeds of inbred lines were produced by self-pollination in order to avoid contamination. Two to three weeks after selfing, bags were removed from selfed ears for proper grain development. Self-pollinated ears (5−10) from each genotype were harvested separately, and seeds were bulked within lines at shelling and stored in darkness at 4.0 °C until grain quality trait measurements, because carotenoids and tocopherols content was affected by storage conditions especially high storage temperature, light, and oxygen (*9, 10*). Carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and total carotenoids), tocopherols (α-tocopherol, δ-tocopherol, γ-tocopherol, total tocopherols, and α/γ tocopherol ratio), starch, oil, protein content, and 100-seed weight (test weight) of each inbred were measured twice during both years.

Oil, Protein, and Starch Measurement. In a 50 mL measuring cylinder, approximately 35 g of representative kernels from each genotype were selected for the measurement of oil, protein, and starch content in VECTER22/N near-infrared analyzer (Bruker Corporation, Germany) (*11*). The same representative sample from each genotype was used to compute 100-seed weight and finally grounded for 120 s in a FW100 Stein mill to further utilize for the detection of carotenoid and tocopherol content.

Carotenoids and Tocopherols Extraction and Measurement. Carotenoids and tocopherols were simultaneously extracted and measured by high-performance liquid chromatography (HPLC) using the method described in ref (*12*). Carotenoid and tocopherol content in each sample were simultaneously separated in an YMC carotenoid C^{30} column (5 μm, 4.6 mm × 250 mm; Waters Chromatography, Milford, MA) connected with a HPLC system of Shimadzu Corporation (Kyoto, Japan) and detected at 450nm and 290nm multiwavelength, respectively. Concentration ranges for carotenoid standards, provitamin A (α-carotene, β-carotene, and β-cryptoxanthin) and non-provitamin A carotenoid (lutein and zeaxanthin) were 0.005−12.5 and 0.024−50 μg/mL, respectively, whereas ranges of α-tocopherol, δ-tocopherol, and γ-tocopherol were 0.073−150, 0.012−25, and 0.15−300 μg/mL, respectively. Total carotenoids and total tocopherols in a respective sample were computed by adding all the individual carotenoids and tocopherols, respectively. The carotenoid standard was extracted and purified in our laboratory (*13*) and is comprehensively outlined in a companion paper (*14*). Commercial standard of tocopherols (α-tocopherol, δ-tocopherol, and γ-tocopherol) were purchased from Sigma (St. Louis, MO). The individual compound was identified in HPLC with the cochromatograph of standard sample of respective compounds.

Statistical Analysis. Analysis of variance and Pearson correlation coefficients for all the traits were performed using the "PROC GLM" procedure of the SAS program (SAS Institute, version 8.0, 1999). Significance of correlation coefficients (r) at $P = 0.05$ or 0.01 is indicated by * or **, respectively. The components of variance, heritability in broad sense, and genetic advance were estimated as explained in ref (*15*). The heritability in broad sense was calculated as

$$h^2B\ (\%) = (\sigma^2g/\sigma^2p \times 100)$$

where σ^2g and σ^2p are genotypic and phenotypic variance, respectively.

Expected genetic advance (GA) was estimated as

$$GA = K(\sigma p)h^2B$$

where K is a standardized selection differential, σp is the phenotypic standard deviation, and h^2B is the broad-sense heritability. Phenotypic (PCV) and genotypic (GCV) coefficients of variation were calculated as

$$PCV = (\sigma p/X) \times 100$$

$$GCV = (\sigma g/X) \times 100$$

where σp, σg, and X are the phenotypic and genotypic standard deviations and the grand mean across years, respectively.

Table 2. Estimate of Variance Components (σ^2p, σ^2g, σ^2ge, σ^2e), PCV (%), GCV (%), h^2B (%), GA, and GA (% of mean) over Two Years (2004 and 2005)[a]

traits	σ^2p	σ^2g	σ^2gy	σ^2e	PCV (%)	GCV (%)	h^2B (%)	GA	GA (%)
β-carotene (μg g^{-1})	0.12	0.10	0.012	0.001	78.75	70.31	79.7	0.58	129.4
β-cryptoxanthin (μg g^{-1})	0.57	0.43	0.07	0.004	86.57	74.62	74.3	1.16	132.5
α-carotene (μg g^{-1})	0.02	0.02	0.002	0.001	127.07	117.16	85.0	0.27	222.5
lutein (μg g^{-1})	11.62	9.39	0.76	0.123	58.74	52.81	80.8	5.68	97.8
zeaxanthin (μg g^{-1})	2.74	2.04	0.19	0.075	54.31	46.83	74.3	2.53	83.2
total carotenoids (μg g^{-1})	22.89	15.73	1.84	0.334	46.47	38.52	68.7	6.77	65.8
α-tocopherol (μg g^{-1})	191.70	163.05	8.29	3.305	57.74	53.25	85.1	24.26	101.2
γ-tocopherol (μg g^{-1})	317.02	286.07	23.75	3.881	53.34	50.67	90.2	33.09	100.6
δ-tocopherol (μg g^{-1})	1.75	1.34	0.29	0.120	60.49	52.86	76.4	2.08	95.2
total tocopherol (μg g^{-1})	484.52	401.19	39.19	7.944	36.96	33.64	82.8	37.55	63.1
α/γ tocopherol ratio	0.80	0.74	0.04	0.006	85.43	82.18	92.5	1.71	162.9
protein (%)	1.67	1.09	0.19	0.062	10.52	8.53	65.6	1.75	14.2
starch (%)	7.61	5.27	1.28	0.657	4.28	3.56	69.2	3.93	6.1
oil (%)	0.57	0.49	0.05	0.024	21.34	19.85	86.5	1.35	38.1
100-seed (g)	20.02	16.81	2.08	1.141	17.37	15.90	83.9	7.74	30.0

[a] σ^2p, phenotypic variance; σ^2g, genotypic variance; σ^2ge, genotype by year variance; σ^2e, error variance; PCV (%), phenotypic coefficient of variability; GCV (%), genotypic coefficient of variability; h^2B (%), heritability in broad sense; GA, genetic advance; GA (%), genetic advance percent.

RESULTS AND DISCUSSION

Traits Variation. Combined analysis of variance (ANOVA) exhibited highly significant differences among the genotypes and genotype by year interactions for all the studied traits (**Table 1**). In the present study, β-carotene, β-cryptoxanthin, α-carotene, lutein, zeaxanthin, and total carotenoids ranged 0.016−1.726, 0.012−3.666, 0.004−0.852, 0.040−17.504, 0.022−6.728, and 0.096−22.495 μg g^{-1} with the joint mean values of 0.449, 0.876, 0.121, 5.803, 3.048, and 10.298 μg g^{-1}, respectively. The pooled mean values of α-tocopherol, γ-tocopherol, δ-tocopherol, and total tocopherols were observed as 23.98, 32.90, 2.189, and 59.55 μg g^{-1} with ranges 3.980−74.81, 2.805−78.16, 0.236−5.338, and 19.60−120.69 μg g^{-1}, respectively. The α/γ-tocopherol ratio ranged from 0.085 to 3.846 with a combined mean value of 1.048. The protein, starch, and oil content varied from 9.584 to 14.757, 57.96 to 70.26, and 2.057 to 5.195% with an average of 12.28, 64.51, and 3.55%, respectively. The variation for 100-seed weight was observed from 17.44 to 37.66 g with a combined mean of 25.78 g. The individual as well as total carotenoids and tocopherols, α/γ-tocopherol ratio, protein, and 100-seed weight were observed highest during 2005. In contrast, only starch and oil content exhibited highest values during 2004.

In general, maize grains have predominant non-provitamin A carotenoids either lutein (*14, 16, 17*) or zeaxanthin (*12*) as compared to provitamin A carotenoids. The current investigated material exhibited lutein as a predominant component of carotenoids. While among tocopherols, several reports have exhibited the preponderance of γ-tocopherol among maize germplasm (*6, 12, 17–20*). The preponderance of γ-tocopherol in this set of 87 inbreds is also in good agreement with the previous studies in maize.

For improvement of any trait, information regarding available variability alone is not sufficient to help the breeders in formulating the breeding strategy. Thus, the expected genetic gain, a prime goal in any breeding program, from a selection can be obtained by the estimate of the coefficient of variability along with heritability. To assess the heritable portion of total variability and in order to compare the variation among various traits, variance components (σ^2p, σ^2g, σ^2ge, σ^2e), phenotypic (PCV) and genotypic coefficient of variability (GCV), heritability in broad sense (h^2B), and expected genetic advance (GA) as percent mean were computed for all traits (**Table 2**). The component of variance clearly indicated that the variability for these traits in this set of material was mainly due to genotypic variance. Carotenoids and tocopherols had the highest, and starch and protein had the lowest coefficient of variation values. The genotypic coefficient of variation (GCV) ranged from 3.56 for starch to 117.16 for α-carotene and exhibited the similar trends as PCV.

Medium (65.6% for protein) to high (92.5% for α/γ tocopherol ratio) level of heritability in broad-sense estimates were observed for all the traits investigated in this experiment (**Table 2**). However, heritability estimates are confined to experimental material and setup and may differ widely in the same crop and same trait (*21*). The heritability estimates observed for these quality traits are in good agreement with results from previous investigations in maize (*19, 22, 23*). We also observed high heritability estimates for carotenoid (84−96%) and tocopherol (79−89%) fractions in separate studies (*14, 18*) whereas medium to high levels of heritability for fractions of carotenoid (48−87%) and tocopherol (62−68%) were reported in refs (*22*) and (*19*), respectively. The GA as percent mean ranged from 6.1% for starch to 222.5% for α-carotene. The higher amount of GA as percent mean for carotenoids and tocopherols suggested the maximum scope of improvements for these nutritionally important compounds. The highest genetic gain observed for α-carotene and the α/γ tocopherol ratio showed that the best genotypes for these traits have extreme variation over population mean (**Tables 1** and **3**).

Relationship among Carotenoids, Tocopherols, Protein, Starch, Oil, and 100-Seed Weight. Understanding the relationship among nutritionally important traits in maize grain with the concomitant goal of improving the end use nutritional value of the maize grain is very important from a breeder's perspective. Pearson correlation coefficients among carotenoids (μg g^{-1}), tocopherols (μg g^{-1}), protein (%), starch (%), oil content (%), and 100-seed weight (g) are given in **Table 4**. At physiological level, the traits studied in this investigation are colocalized (endosperm and/or embryo of seed), whereas at the biochemical level, some of the traits are part of the same biosynthetic pathway such as carotenoids and tocopherols. Thus, the relationship between levels of an individual compound is highly relevant from breeding strategy perception.

β-Carotene, a most important provitamin A carotenoid that yields two molecules of retinal, was observed significantly positive correlated with both β-cryptoxanthin and zeaxanthin. A highly positive association was also found between β-cryptoxanthin and zeaxanthin. On the other hand, the most abundant carotenoid (lutein) detected in this study showed significant and

Table 3. List of Best Performing Genotypes along with Heterotic Groups for Individual Traits

HG	inbred	β-carot	β-crypt	α-carot	lut	zeax	TC	α-toco	γ-toco	δ-toco	TT	α/γ toco	P	S	O	100-SW
Tang SPT	3H2	1.73[a]	3.67[a]	0.02	4.90	5.76	16.08	25.03	18.96	1.45	45.3	1.32	11.89	66.56	4.72	19.79
Tang SPT	K12	0.46	0.52	0.17	10.80	4.24	16.19	21.32	4.31	5.34[a]	32.5	3.44	11.56	70.26[a]	2.23	29.74
Zi330	Si434	0.53	0.80	0.47	17.50[a]	3.19	22.50[a]	13.53	54.82	3.59	73.2	0.24	13.88	62.19	2.66	21.98
Tang SPT	TianYaSi	0.14	0.27	0.09	4.22	2.48	7.19	23.02	25.94	1.09	50.7	0.89	14.76[a]	63.03	2.06	24.36
Reid	K22	1.05	1.10	0.04	5.12	6.73[a]	14.03	21.92	8.45	2.73	34.2	2.26	12.72	61.79	3.76	25.20
Reid	Hu803	0.29	0.39	0.10	8.98	4.03	13.79	74.81[a]	44.08	1.88	120.7[a]	1.70	13.37	60.82	4.28	29.08
Reid	7884	0.13	0.40	0.16	7.50	2.04	10.22	17.33	78.16[a]	1.44	97.6	0.22	11.13	62.99	3.40	32.54
Reid	Ye832	0.13	0.42	0.31	7.43	2.33	10.62	47.83	42.93	2.53	92.0	1.15	10.24	67.85	5.20[a]	26.18
Reid	Ye107	0.49	1.62	0.00	2.64	5.60	10.36	5.70	39.37	0.38	44.5	0.15	12.97	61.86	2.27	37.66[a]
Reid	Dan9046	1.10	0.99	0.85[a]	7.91	1.39	12.24	30.27	12.68	3.68	47.5	2.26	13.36	64.75	3.47	31.84
other	Qi205	0.04	0.13	0.02	5.66	1.77	7.62	24.04	4.44	2.64	32.9	3.85[a]	11.74	62.35	5.04	17.44

[a] Indicates the best performance of that genotype for concerned trait: HG, heterotic group; β-carot, β-carotene; β-crypt, β-cryptoxanthin; α-carot, α-carotene; lut, lutein; zeax, zeaxanthin; TC, total carotenoids; α-toco, α-tocopherol; γ-toco, γ-tocopherol; δ-toco, δ-tocopherol; TT, total tocopherols; α/γ toco, α/γ tocopherol ratio; P, protein; S, starch; O, oil content; 100-SW, 100-seed weight.

Table 4. Pearson Correlation Coefficients among Carotenoids (μg g^{-1}), Tocopherols (μg g^{-1}), Protein (%), Starch (%), Oil (%), and 100-Seed Weight (g) in Maize Grains of 87 Elite Maize Inbreds[a]

	lut	zeax	β-cry	α-car	β-car	TC	δ-toc	α-toc	γ-toc	TT	AGR	P	S	O	TW
lut	1														
zeax	0.224[b]	1													
β-cry	−0.116	0.611[c]	1												
α-car	0.345[c]	−0.167	−0.078	1											
β-car	0.151	0.560[c]	0.711[c]	0.114	1										
TC	0.851[c]	0.671[c]	0.350[c]	0.236[b]	0.519[c]	1									
δ-toc	0.630[c]	0.228[b]	−0.144	0.159	0.119	0.555[c]	1								
α-toc	0.110	0.044	−0.091	−0.019	0.014	0.085	0.210	1							
γ-toc	0.186	−0.034	−0.043	0.074	−0.117	0.117	−0.145	−0.112	1						
TT	0.266[b]	0.007	−0.103	0.070	−0.079	0.185	0.065	0.556[c]	0.760[c]	1					
AGR	−0.060	0.017	−0.054	−0.036	0.080	−0.044	0.295[c]	0.498[c]	−0.744[c]	−0.278[c]	1				
P	−0.031	0.048	0.102	0.118	0.158	0.027	−0.058	−0.114	−0.018	−0.09	−0.031	1			
S	−0.105	−0.219[b]	−0.177	0.004	−0.169	−0.201	−0.074	0.014	−0.020	−0.006	−0.009	−0.609[c]	1		
O	0.026	−0.078	0.096	0.005	0.038	0.011	0.059	0.185	0.182	0.274[b]	−0.022	−0.302[c]	0.146	1	
TW	0.189	0.131	−0.145	0.073	−0.052	0.166	0.179	0.155	−0.017	0.098	0.062	−0.080	−0.051	−0.296[c]	1

[a] Significance of the correlation coefficients at the 5% or 1% level is indicated by Note: Lut, lutein; Zeax, zeaxanthin; β-cry, β-cryptoxanthin; α-car, α-carotene; β-car, β-carotene; TC, total carotenoids; δ-toc, δ-tocopherol; α-toc, α-tocopherol; γ-toc, γ-tocopherol; AGR, α/γ tocopherol ratio; TT, total tocopherols; P, protein; S, starch; O, oil; TW, 100-seed weight. [b] Significance of the correlation coefficients at the 5% level. [c] Significance of the correlation coefficients at the 1% level.

positive correlation with α-carotene. Furthermore, one significant but lower level of positive association was also found between non-provitamin A carotenoids (lutein and zeaxanthin). All the individual carotenoids exhibited significantly positive correlation with total carotenoids. In the past, significant positive correlations between β-carotene and β-cryptoxanthin and β-cryptoxanthin and zeaxanthin have been also found (*16*). There was no correlation between carotenoids and oil content detected in this investigation, and this is also earlier reported (*24*), whereas there is a weak but significantly negative association observed between zeaxanthin and starch content.

The correlation study did not detect any relationship among individual tocopherols, although total tocopherols showed highly significant and positive correlation with α-tocopherol and γ-tocopherol while no association was observed with δ-tocopherol. Earlier researchers also did not find any associations among these individual tocopherols expect γ-tocopherol and δ-tocopherol (*20*). The δ-tocopherol showed significantly positive correlation with lutein, zeaxanthin, and total carotenoids. The α/γ-tocopherol ratio showed significantly positive correlation with δ-tocopherol and α-tocopherol, whereas γ-tocopherol and total tocopherols exhibited negative correlation. In addition, a lower but statistically significant level of positive correlation was also found between lutein and total tocopherols.

Oil content was found significantly positive correlated with total tocopherols whereas no association was found with starch. Some authors reported from insignificant (*19*) to positively significant (*25*) association between oil content and tocopherol. No significant correlation was found between protein and 100-seed weight, but both were significantly negative correlated with oil content. A negative correlation between oil content and 1000-grain weight also was reported in ref (*26*). As expected, a strong negative correlation was detected between starch and protein content. A high degree of association in the negative direction between starch and protein content in maize is frequently found in the literature, suggesting an unlikely simultaneous selection for both traits (*23, 27, 28*).

Elite Heterotic Groups and Genotypes for Nutritional Traits. In this set of material, significant differences were observed among heterotic groups for these nutritional traits. Among major heterotic groups, Reid identified as a best source for tocopherols including high levels of α-tocopherol, γ-tocopherol, and total tocopherols, and the best performing genotypes identified in this investigation for these tocopherols were also from Reid (**Table 3**). For carotenoids, Zi330 showed high mean performance for β-carotene, lutein, and total carotenoids (see Supporting Information), but the best genotypes observed for provitamin A carotenoids did not correspond to Zi330. Elite genotypes that have been identified as a genetic source for enhanced levels of these traits did not exhibited a high amount of all the individual fractions of carotenoid and/or tocopherol (**Table 3**).

Prospects for Biofortification with Improved Nutritional Values of Maize Grain. The prime goal of maize breeders is always yield enhancement, but views of improving the livelihoods through increased levels of micronutrients especially

provitamin A carotenoids in food is highly warranted. Thus, high GA as percent mean along with wide variation and high heritability estimates observed for these nutritionally important traits indicated the considerable scope for improving the nutritional status of commercial maize cultivars. Most of traits studied in this experiment were either significantly positive correlated or independent except a few traits (**Table 4**). However, we did not measure the grain yield in this investigation, but previous results in maize showed positive correlation of grain yield with total carotenoids (*12*). Thus, correlated response of these traits revealed by inter-relationship study may help the maize breeders to formulate their breeding strategy for the enrichment of maize cultivars with improved nutritional level. The results observed in this study indicate that the amount of provitamin A carotenoids in commercial maize hybrids in China is very low, because for carotenoids including provitamin A carotenoids, additive gene action predominate in maize (*12*) and inbreds studied in the present investigation were used extensively in the Chinese maize breeding program (*8*).

HarvestPlus (www.harvestplus.org), a research program implemented with the international research institutes of the CGIAR, concentrating on biofortification of staple food crops, fixed the final target of 15 $\mu g\ g^{-1}$ provitamin A carotenoids (8.5 $\mu g\ g^{-1}$ for interim target) for maize on the assumption of 50% retention during processing and cooking, a 12:1 bioconversion rate of β-carotene to retinol, 200 and 400 g daily maize consumption by children and women, respectively (*29, 30*). Recently, high bioconversion ratio (from 12:1 μg to 2.8:1 μg) for provitamin A carotenoid from maize to vitamin A has been observed (*31*), However, the best genotype identified for provitamin A carotenoids in this investigation showed 3.58 $\mu g\ g^{-1}$ provitamin A content [β-carotene + (β-cryptoxanthin + α-carotene/2)] and seems to be too low to be a dietary source of this vitamin (**Table 3**). But recently, two elite inbreds (C1.7 and Deexp3) having a high amount of β-carotene (13.6 $\mu g/g$) have been identified in temperate germplasm, and the molecular marker for β-carotene content in these inbreds has been developed (*30, 32*). Thus, these inbreds could be utilized in the breeding program by marker assisted selection for enhancement of provitamin A content in locally adapted elite germplasm in China.

In addition to improving the provitamin A contents in major food crops, bioavailability of these compounds has also emerged as a striking issue for nutritional science especially in maize. Thus, the simultaneous breeding for multinutritional traits in maize not only has direct beneficial effects in fighting particular deficiencies such as VAD but also has several indirect advantages such as enhancement in bioavailability of provitamin A carotenoids, oil stability, and protection against several other diseases in many low income families especially in developing countries that exist on a simple plant based diet composed primarily of maize. Thus, findings of this experiment may help the biofortification of maize grain with an enhanced level of carotenoids, tocopherols, oil, protein, and starch content.

ABBREVIATIONS USED

VAD, vitamin A deficiency; HPLC, high-performance liquid chromatography; σ^2p, phenotypic variance; σ^2g, genotypic variance; σ^2ge, genotype by year variance; σ^2e, error variance; PCV, phenotypic coefficient of variability; GCV, genotypic coefficient of variability; h^2B, heritability in broad sense; GA, genetic advance; GA (%), genetic advance percent; CGIAR, Consultative Group on International Agricultural Research.

ACKNOWLEDGMENT

The authors would like to thank Delia B. Rodriguez-Amaya for technical assistance during the extraction of carotenoids standard.

Supporting Information Available: Tables listing information about the 87 elite maize inbreds and concerning the performance of nutritional traits in heterotic groups and maize genotypes. This material is available free of charge via the Internet at http://pubs.acs.org.

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Received for review December 31, 2007. Revised manuscript received June 7, 2008. Accepted June 8, 2008. We acknowledge the Indian and Chinese governments for providing the fellowship to the first author. This work was supported by HarvestPlus and National Nature Science Foundation of China.

JF7037967