Cottonseed Protein, Oil, and Mineral Nutrition in Near-Isogenic *Gossypium hirsutum* Cotton Lines Expressing Leaf Color Phenotypes under Field Conditions

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Abstract

Information about the effects of phenotype traits on cottonseed protein, oil, and nutrients is scarce. The objective of this research was to investigate the effects of leaf color trait on seed nutrition in near-isogenic *Gossypium hirsutum* cotton expressing green (G) and yellow (Y) leaf color phenotypes. Our hypothesis was that leaf color can influence the accumulation of nutrients in seeds. Sets of isogenic lines were: DES 119 (G) and DES 119 (Y); DP 5690 (G) and DP 5690 (Y); MD 51ne (G) and MD 51ne (Y); SG 747 (G) and SG 747 (Y). Each NIL set is 98.44 % identical. Parent line SA 30 (P) was used as the control. The experiment was repeated for two years (2014 and 2015). The results showed that, in 2014, seed oil in DES 119 (G) and SG 747 (G) were significantly higher than their equivalent yellow lines. Green lines showed higher content of phosphorus compared with yellow lines. Higher levels of Cu, Fe, Mn, Ni, and Zn were recorded in DES 119 (G) and MD 51ne (G). In 2015, seed protein, oil, C, N, P, B, Cu, and Fe were higher in green lines than in yellow lines. There was a significant correlation between protein and nutrients, and between oil and nutrients in 2015, but not in 2014 as the temperature was warmer in 2015 than in 2014. This research demonstrated that leaf color can alter seed composition and mineral nutrition under certain environmental growing conditions such as temperature.

Keywords

Isogenic Cotton, Cottonseed, Seed Protein, Seed Oil, Seed Composition

1. Introduction

Cotton is a major crop in the world [1] and cottonseed is a major source of fiber, oil, proteins, and considered as a nutritious ingredient in animal feed and human food products [2] [3] [4]. In addition to its content of protein and oil [5], cottonseed contains minerals [1] [6], and is used as renewable biofuel [7]. A cotton plant produces about 1.6 kg of seed for every kg of lint, and after ginning, the seeds are either used as animal feed or processed to products, including oil, meal, hull and short linter fibers [8] [9]. Because of the high content of protein and oil in seed (about 17% - 27% oil and 12% - 32% protein) [1] [10], cotton is the fifth largest oil crop in the world and is the second largest potential source of plant protein. Due to the large demand for cottonseed fiber by the textile industry, cottonseed quality has been relatively neglected, and as a result, narrowing the genetic variation of cotton for seed quality improvement through breeding. Therefore, a deeper understanding of metabolic processes that determine the storage reserves in seeds is critical for higher seed yield and quality. The seed and its embryo are the sites of accumulation of storage reserves and secondary metabolites. Recent research revealed biochemical, cellular, and molecular mechanisms underlying biogenesis of seed storage compounds and secondary metabolites, and identified several genes involved in these processes that were essential for our understanding in improving nutritional seed qualities and enhanced functional properties of seeds [4]. However, in spite of novel approaches used by researchers, including agricultural practices and integrated management strategies [4] [6] [7]; traditional and molecular genetics [4]; quantitative trait loci mapping [1]; transformation [11]; transcriptome, molecular biology, and physiology [12]; functional genomics [13]; photosynthesis and carbon metabolism [4] [14] [15]; and gene modification and gene silencing [16] [17], achieving higher yield is still challenging, and information to select for higher cottonseed nutritional qualities is still scarce. Maintaining higher cottonseed production, fiber quality, and seed nutritional qualities is critical for textile, human nutrition, and meal industries.

The predominant cotton species grown in the USA is upland cotton, *Gossypium hirsutum* [18] [19] [20]. Other species, including *G. barbadense*, *G. arboreum*, and *G. herbaceum* are also grown and represent 5% worldwide production [13]. Breeding for cotton yield and fiber quality has been the major goal of researchers. However, it has been reported that the selection for higher yield, due to increased dry matter in the reproductive growth [14] [21], will be inefficient due to the fact that the photosynthesizing leaf area becomes the major limiting factor [14]. Therefore, it was concluded that the variation of photosynthetic components had a narrow range, making it challenging to choose for superior breeding lines [22]. Although serious attention was given to new approaches for higher yield and seed nutritional qualities, for example, cotton genome research [16] [17], cotton breeding [1], sustainable agricultural practices [23], and agronomic strategies [24] [25], achieving higher yield became chal-

lenging due to lower use efficiency [26] [27] [28] [29] is still the main challenge worldwide [7].

Based on the above reports, new tools need to be discovered to achieve higher yield and higher seed nutritional qualities [30]. For example, previous research used cotton mutants and isogenic breeding lines to show their significant role in understanding fiber development [31] [32], yield [20], and cottonseed protein, oil, and mineral nutrition [33] [34]. Our focus in this research was to use a novel approach to understand the effects of leaf color on cottonseed composition constituents (protein and oil), N, S, and mineral nutrients using four sets of near-isogenic breeding lines, expressing leaf color phenotypes under Mississippi Delta conditions.

2. Materials and Methods

2.1. Development of Near-Isogenic Lines

The details of NILs development was reported elsewhere [35]. Briefly, the NILs were developed by the standard backcross breeding method. The NILs were developed for virescent (yellow) and wild-type (green) leaf sets from four modern upland cotton genotypes. The four modern genotypes, selected in 1995 as the wild-type parents, were cultivars DP5690 (Monsanto Company, St. Louis, MO, USA; PVPC 009100116), SG747 (Monsanto Company, St. Louis, MO, USA; PVPC 009800118), DES 119 (Mississippi Agricultural and Forestry Experiment Station, Mississippi State, MS, USA; PI 606809; PVPC 008500176) and MD51ne (USDA-ARS, Stoneville, MS, USA; PI 566941). The parent plants were selfed through 9 generations using single seed descent in insect-controlled greenhouses and using selfing bags in the field placing them over flower buds before flowering. The F1 seeds from each cross were grown and self-fertilized and the F2 seed were then planted and the segregating yellow plants were used in the backcrossing events. Then the parent lines were used to cross once and proceed to the F2 generation then begin the BC1F1 to BC5F1 with the subsequent BC1F2 to BC5F2 generations being produced in the greenhouse during fall and winter. After six years of crossing/back crossing, BC5F2, NILs expressing the two leaf colors were selected for the four upland cotton genotypes. Therefore, after the 11 years of development, we then had to increase seed for 2 years by bag-selfing using the greenhouse. Theoretically, each NIL set was 98.44% identical. Seed increases were obtained from individual plants by self-pollination in the greenhouse or in the field. Pollinating insects were prevented by the use of screens and insecticides, and cross-pollination of field grown flowers was avoided by placing 10.2 × 15.2 cm organza bags (Gifts International, Ontario, CA) over the flower buds prior to opening to prevent pollinators. Validation occurred in a previous paper [35], using an obsolete verses modern cotton line study. The SA 30 line had large bolls being a derivative of Mebane type cotton. One of the observations for modern line cotton is that they have smaller bolls than the obsolete lines and in all the modern backgrounds the yellow lines produced smaller bolls with

higher total yields. This is an indication of "changing the genotype" from the Mebane type cotton to predominantly a modern line cotton with their respective yield and fiber qualities resembling the parental modern lines. Two groups [36] [37] have mapped this gene and reported the data and what gene it is supposed to be; a single modification from arginine (Arg) to lysine (Lys) in a protein. Sequencing of NILs has taken place using the Ligon lintless lines and the gene for Ligon lintless was identified using Li1 and DP 5690 markers.

2.2. Field Management and Growth Conditions

A field experiment was conducted in 2014 and repeated in 2015 in Stoneville, MS. The near-isogenic lines for virescent (yellow) and wild-type (green) leaf sets were developed by a backcross breeding method using four modern upland cotton varieties (line development was detailed by others) [35]. The four modern upland cotton genotypes/cultivars were selected in 1995, and were: DP 5690 (Monsanto Company, St. Louis, MO, USA; PVPC 009100116), SG 747 (Monsanto Company, St. Louis, MO, USA; PVPC 009800118), DES 119 (Mississippi Agricultural and Forestry Experiment Station, Mississippi State, MS, USA; PI 606809; PVPC 008500176) and MD 51ne (USDA-ARS, Stoneville, MS, USA; PI 566941). These parental lines were self-pollinated to develop pure inbred lines by single seed descent selection through nine generations in greenhouse and field at the USDA-ARS in Stoneville, MS. The virescent line SA 30 (PI 528447) was used as a parent (SA 30 was obtained from the Mississippi Obsolete Variety Collection [36]. After six years of crossing, the following four sets of BC_5F_2 NILs expressing the leaf color (green, G; yellow, Y) were developed: DES 119 (G) and DES 119 (Y); DP 5690 (G) and DP 5690 (Y); MD 51ne (G) and MD 51ne (Y); SG 747 (G) and SG 747 (Y). Each NIL set was theoretically 98.44% identical. The green NILs with the parent SA 30 were planted at Stoneville, MS, the USA in 2014 and repeated in 2015 as described in detail elsewhere [35]. Briefly, plants were planted in four rows spaced of 1.02 m apart. Each plot was 9.14 m long with a 3.04 m alley between plots. The soil was a Bosket very fine sandy loam soil (fine-loamy, mixed, active, thermic Mollic Hapludalfs). Plots were managed in the season following recommended agronomic and pest control practices for the state of Mississippi (Mississippi State University Extension). Planting dates were May 5, 2014 and April 30, 2015, and harvesting dates were October 9, 2014 and October 8, 2015. Soil and leaf samples were taken before first square to check the nutrient levels in soil and leaves. Mature seeds were hand harvested, ginned, and delinted prior to seed composition and mineral nutrition analyses as described below.

2.3. Soil Nutrients Analysis

Soil analysis for nutrient levels in soil was conducted by inductively coupled plasma spectrometry (Thermo Jarrell-Ash Model 61E ICP and Thermo Jarrell-Ash Autosampler 300 (C Jarrell-Ash Corporation, Waltham, MA, USA) as

previously detailed [38]. Briefly, the analysis was conducted on 5 g soil:20 ml Mehlich-1 solution. Analysis of N, S, and C were based on the Pregl-Dumas method [39] [40] [41] using a C/N/S elemental analyzer with thermal conductivity cells (LECOCNS-2000 elemental analyzer, LECO Corporation, St. Joseph, MI, USA). Oxygen atmosphere at 1350˚C was used to combust soil samples and to convert elemental N, S, and C into N_2, SO_2, and CO_2 gases. The content of N, S, and C in soil was analyzed by the elemental analyzer as previously detailed [38]. Soil analysis showed no soil or leaf nutrient deficiencies. The following are averages of nutrient content in soil across the field: C = 0.92%, N = 0.092%; and (g·kg^{-1}) P = 0.355, K = 2.27, S = 0.091, Ca = 3.5, Mg = 3.0, and Fe = 18.7; and (mg·kg^{-1}) B = 2.2, Cu = 13.0, Zn = 57.5. Organic matter in soil was 3.3%. The crop did not show any nutrient deficiency symptoms under these conditions.

2.4. Analysis of Seed Minerals, N, S, and C

Nutrient content in seed was determined in the ground, dried samples. Samples were ground with a Laboratory Mill 3600 (Perten, Springfield, IL, USA) and analyzed by digesting a 0.6 g in HNO_3 in a microwave digestion system and nutrients were quantified using inductively coupled plasma spectrometry (Thermo Jarrell-Ash Model 61E ICP and Thermo Jarrell-Ash Autosampler 300) [38]. Seed N, C, and S were determined on 0.25 g samples by the C/N/S elemental analyzer as detailed previously [38] [42].

2.5. Determination of Seed B, Fe and P

Boron concentration in mature seeds was determined by the azomethine-H method [43] [44]. Briefly, seed samples were ground in a Laboratory Mill 3600 (Perten, Springfield, IL, USA) and a 1.0 g sample was combusted to ash at 500˚C and extracted with 20 ml of 2 M HCl at 90˚C for 10 min. The mixture was filtered and a 2 ml sample added to 4 ml of buffer solution containing 25% ammonium acetate, 1.5% EDTA, and 12.5% acetic acid. A freshly prepared solution (4 ml) of 0.45% azomethine-H in 1% of ascorbic acid [45] was added, and the B concentration was measured at 420 nm using a Beckman Coulter DU 800 spectrophotometer (Beckman Coulter, Inc., Brea, CA, USA). Iron concentration in mature seeds was determined according to established methods [46] [47]. Briefly, seed samples were ground in a Laboratory Mill 3600 (Perten, Springfield, IL, USA) as described above. Then, samples were digested with hydrochloric acid (109 ml of 3% w/w) and extracted. The concentration of Fe was determined based on the color complex reaction between ferrous Fe with 1,10-phenanthroline as described by others [38] [42]. Phenanthroline reagent solution of 0.25% (w/v) in 25% (v/v) ethanol and quinol solution (1% w/v) were prepared, and the concentrations of Fe ranging from 0.0 to 4 µg·ml^{-1} of Fe in 0.4 M HCl were made for the standard curve. Iron concentration was measured by a Beckman Coulter DU 800 spectrophotometer at an absorbance of 510 nm as previously described by others [38] [42]. Phosphorus concentration in mature seeds was determined by the

yellow phosphor-vanado-molybdate complex method [48], and as previously described [38] [42]. The P was extracted with 2 ml of 36% v/v HCl. A reagent of 5 ml of 5 M HCl and 5 ml of ammonium molybdate-ammonium metavanadate was used. The concentration of P was determined by reading the absorbance at 400 nm using a Beckman Coulter DU 800 spectrophotometer as previously described by others [38] [42].

2.6. Cottonseed Protein and Oil Analysis

Mature seeds were collected from each plot and analyzed for protein and oil content. Approximately 25 g of seed was ground using a Laboratory Mill 3600 (Perten, Springfield, IL, USA). The content of protein and oil in cottonseed were analyzed by near infrared reflectance [33], using a diode array feed analyzer AD 7200 (Perten, Springfield, IL, USA). Calibrations were developed using Perten's Thermo Galactic Grams PLS IQ software, and the calibration equation was established according to AOAC methods [49] [50]. Protein and oil were expressed on a seed dry matter basis [33] [34].

2.7. Experimental Design and Statistical Analyses

The experiment was a randomized complete block design with three replications. The main plot treatment was a line (four sets of NILs and the virescent parent SA 30 were used). Statistical analyses were performed using PROC MIXED (SAS, SAS Institute, 2002-2010) [51]. Replicate (Rep) within year, and treatment by replicating within year [(Rep (Year); T × Rep (Year)] were considered as random effects. Year and line were modeled as fixed effects. Residuals of the random effect factors are shown as covariance parameters in the tables. The residuals refer to Restricted Maximum Residual Likelihood (REML) values, which reflect the total variance of the random parameters in the model [34]. Means were separated using Fisher's protected least significant difference test at significant level of 5% using SAS (SAS Institute, 2002-2010) [51]. Correlations were conducted using Proc Corr in SAS. Since year by line interactions were significant for some seed composition components and mineral nutrition, results were presented separately in each year.

3. Results

3.1. Analysis of Variance

Analysis of variance showed that there was a significant effect of year for protein, but no significant differences of line or year by line interaction for protein (Table 1). There was a significant effect of year and line for oil, but no significant differences of year by line interactions for oil. Significant effects of year, line, and their interaction for C, N, P, B, Cu, Fe, and Mn content were observed. Only the line by year interaction was significant for S. Potassium was affected only by year. Calcium was affected by year and line, but no significant effects of year by line interaction was observed. Both Ni and Zn were significantly affected by line and year by line interactions.

Table 1. Analysis of variance of the effects of year, line, and their interactions for seed composition (%: protein and oil; N, C, S, P, K, Ca (%); B, Cu, Fe, Mn, Ni, and Zn (mg·kg^{-1}) in near-isogenic cotton lines expressing green or yellow leaf color and their virescent parent. The experiment was conducted in Stoneville, MS in 2014 and 2015.

Effect		Protein		Oil		C		N		S		P		K	
	DF	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Year	1	10.43	**	6.63	*	45.41	***	36.05	***	3.39	NS	148.58	***	4.91	*
Line	8	1.90	NS	3.69	**	18.68	***	18.54	***	1.95	NS	24.30	***	0.99	NS
Year * Line	8	1.87	NS	1.07	NS	13.88	***	3.81	***	5.02	***	2.25	*	1.64	NS
Residuals		5.16		4.80		0.67		0.11		0.0012		0.0018		0.02	
Effect		Ca		B		Cu		Fe		Mn		Ni		Zn	
Year		22.56	***	73.79	***	5.66	*	122.38	***	67.39	***	2.05	NS	4.27	NS
Line		16.66	***	8.19	***	12.55	***	42.48	***	23.64	***	15.56	***	51.42	***
Year*Line		0.41	NS	2.64	*	5.50	***	1.11	*	3.88	***	7.59	***	2.56	*
Residuals		0.0001		0.47		0.51		8.39		0.52		0.17		2.18	

*Significant at $P \leq 0.05$; **significant at $P \leq 0.01$; ***significant at $P \leq 0.001$.

3.2. Effects of Leaf Color on Seed Protein, Oil, C, N, S, and Minerals

In 2014, the mean value showed that leaf color did not have a clear pattern effect on seed protein (Table 2). Seed oil in SG747 (G) was significantly higher than the SG 747 (G). The parent (P) line (SA 30) [(SA 30 (P)], control, was also competitive (28.90%) compared with other lines. Since the oil and protein generally have an inverse correlation, protein in some lines generally showed the opposite trend. Carbon content in DES 119 (G), DES 119 (Y), and DP 5690 (G) was significantly higher than DP 5690 (Y), SA 30 (P), MD 5ne (G), MD 5ne (Y), SG 747 (G), and SG 747 (Y); lower levels of C were recorded in SG 747 (G) and SG 747 (Y). Nitrogen content in DES 119 (G), DES 119 (Y), DP 5690 (G), MD 51ne (G), SG 747 (G), and SG 747 (Y) was higher than DP 5690 (Y), SG 747 (G), and SG 747 (Y), but the lowest N content was recorded in DP 5690 (Y). Sulfur content in DES 119 (G),

DES 119 (Y), SG 747 (G), and SG 747 (Y) were higher than DP 5690 (G), DP 5690 (Y), and MD 51ne (G), and lower levels of S content were recorded in these lines. Calcium was higher in all G lines than their equivalent Y lines. The parent line, SA 30 (P), also showed higher Ca content than DES 119 (Y), DP 5690 (G), DP 5690 (Y), MD 51ne (Y), and SG 747 (Y). Potassium did not show variability among the lines, except for DP 5690 (Y), where the lowest K content was observed. Potassium content in DP 5690 (G) was higher than in its equivalent line DP 5690 (G). Green lines showed higher content of P compared to yellow lines, including the SA 30 (P), and high levels of P content were recorded in DES 119 (G) and DP 5690 (G), and low levels of P content were in SA 30 (P) and MD 51ne (Y). Boron content was the highest in DES 119 (G), but the lowest in DP 5690 (Y), and the rest of the lines, including SA30 (P), showed an intermediate level of B concentration. Except for MD 51ne (G), all G lines showed higher B

Table 2. Effect of leaf color (green, G; and yellow, Y) on seed composition (protein and oil expressed as %), and nutrients (N, C, S, K, P, and Ca were expressed as %; B, Cu, Fe, Mn, Ni, and Zn were expressed as mg·kg⁻¹) in four sets of near-isogenic cotton lines and their virescent parent grown in Stoneville, MS in 2014.

Line	Protein	Oil	C	N	S	Ca	K
DES 119 (G)	21.44	29.92	55.41	4.37	0.48	0.17	1.27
DES 119 (Y)	20.42	28.58	55.41	4.42	0.48	0.14	1.22
DP 5690 (G)	22.88	29.90	56.85	4.70	0.37	0.14	1.27
DP 5690 (Y)	21.84	29.06	54.74	3.20	0.37	0.11	0.85
MD 51ne (G)	22.80	27.52	54.88	4.40	0.37	0.17	1.27
MD 51ne (Y)	23.21	27.37	54.99	3.33	0.41	0.15	1.24
SA30 (P)	23.94	28.90	54.42	3.40	0.41	0.16	1.24
SG 747 (G)	23.26	30.16	53.82	4.28	0.43	0.17	1.16
SG 747 (Y)	25.42	28.28	53.84	4.52	0.45	0.14	1.19
LSD	1.62	1.61	0.20	0.08	0.02	0.007	0.12
	P	B	Cu	Fe	Mn	Ni	Zn
DES 119 (G)	0.91	13.55	11.18	77.76	17.45	3.44	46.13
DES 119 (Y)	0.77	11.57	9.57	64.63	14.10	2.23	44.93
DP 5690 (G)	0.87	12.40	8.52	53.33	15.79	4.43	35.48
DP 5690 (Y)	0.73	10.92	8.38	55.04	12.45	2.93	36.03
MD 51ne (G)	0.84	12.00	11.73	68.91	16.93	4.34	45.68
MD 51ne (Y)	0.74	12.37	7.57	55.09	14.50	1.79	45.53
SA30 (P)	0.76	12.02	8.95	55.47	14.13	2.62	41.50
SG 747 (G)	0.80	12.90	9.30	67.10	16.95	3.40	44.44
SG 747 (Y)	0.73	12.72	9.60	57.02	13.63	2.27	43.60
LSD[a]	0.02	0.36	0.40	1.12	0.38	0.22	0.81

[a]Fisher's protected least significant difference (LSD) test at the 5% level of significance.

compared with their equivalent Y lines, although the difference in B content between SG 747 (G) and SG 747 (Y) was not significantly different. The content of Cu and Fe were the highest in both DES 119 (G) and MD 51ne (G). For Mn, SG 747 (G) is higher than MD 51ne (G); for Ni, DP 5690 (G) was higher than MD 51ne (G), and SG 747 (G) is equivalent to DES 119 (G); for Zn, MD 51ne (Y) is equivalent to MD 51ne (G) and is among the highest levels. With few exceptions, the lower concentration of these nutrients (Cu, Fe, Mn, Ni, and Zn) was recorded in the yellow lines, including the parent line SA 30 (P). The range of nutrient content in seed in 2014 was (%) from 20.42 to 25.42 (24.5% difference) for protein; 27.37 to 30.16 for oil (10.2% difference); 53.82 to 56.85 for C (5.6% difference); 3.2 to 4.70 for N (47% difference); 0.11 to 0.17 for Ca (55% difference); 0.85 to 1.27 for K (49% difference); 0.73 to 0.91 for P (25% difference) and (mg·kg⁻¹) from 10.92 to 13.55 for B (24% difference); 53.33 to 77.76

for Fe (46% difference); 12.45 to 17.45 for Mn (40% difference); 1.79 to 4.43 for Ni (47% difference); and 35.48 to 46.13 for Zn (30% difference).

In 2015, protein content was higher in all G lines than Y lines (Table 3). Oil content was also significantly higher in all G lines and lower in Y lines. The parent line SA 30 (P) had also lower oil content. The content of C and N was higher in all G lines than in Y lines, and the lowest N content was recorded in SA 30 (P). No clear trend was observed for S and K, but S content was higher in DP 5690 (Y), SG 747 (Y), and MD 51ne (Y), and SA 30 (P) than their equivalent G lines. Calcium had no clear trend as well, but Ca content was higher in DES119 (G), MD51ne (G), MD51ne(Y), SA30 (P), and SG747 (G) than the rest of the lines. The content of P, B, Cu, and Fe was higher in G lines than their equivalent Y lines and higher than SA 30 (P). A similar trend was noticed for Mn, except for SG 747 (G) and SG 747 (Y), where both lines did not show any significant differences

Table 3. Effect of leaf color (green, G; and yellow, Y) on seed composition (protein and oil expressed as %), and nutrients (N, C, S, K, P, and Ca were expressed as %; B, Cu, Fe, Mn, Ni, and Zn were expressed as $mg \cdot kg^{-1}$) in four sets of near-isogenic cotton lines and their virescent parent grown in Stoneville, MS in 2015.

Line	Protein	Oil	C	N	S	Ca	K
DES 119 (G)	27.23	31.70	57.40	4.23	0.44	0.15	1.20
DES 119 (Y)	23.30	25.70	51.67	3.07	0.37	0.12	1.07
DP 5690 (G)	26.87	30.53	54.50	4.07	0.42	0.13	1.13
DP 5690 (Y)	23.43	26.60	51.93	3.30	0.49	0.10	1.17
MD 51ne (G)	25.43	26.47	56.43	4.00	0.43	0.14	1.04
MD 51ne (Y)	21.47	24.40	50.63	2.67	0.45	0.14	1.07
SA30 (P)	23.10	24.90	51.77	2.50	0.45	0.16	1.04
SG 747 (G)	27.27	29.27	55.43	4.47	0.43	0.15	1.08
SG 747 (Y)	25.07	26.30	51.07	3.40	0.47	0.13	1.05
LSD	0.80	0.67	0.62	0.25	0.02	0.0047	0.03
	P	B	Cu	Fe	Mn	Ni	Zn
DES 119 (G)	0.82	11.73	10.73	64.07	15.17	3.27	45.57
DES 119 (Y)	0.68	9.14	9.63	55.47	12.67	2.33	44.87
DP 5690 (G)	0.72	11.43	11.63	44.97	12.80	3.17	33.23
DP 5690 (Y)	0.55	10.00	9.61	42.77	10.80	4.03	35.80
MD 51ne (G)	0.70	11.73	10.80	52.73	13.37	4.01	47.27
MD 51ne (Y)	0.54	9.43	8.53	45.13	10.93	3.80	40.77
SA30 (P)	0.51	10.23	9.13	43.80	12.20	2.97	37.93
SG 747 (G)	0.70	11.90	10.60	55.40	13.13	3.20	43.13
SG 747 (Y)	0.55	10.37	8.27	47.73	13.23	2.13	42.77
LSD[a]	0.02	0.42	0.40	2.11	0.48	0.25	0.99

[a]Fisher's protected least significant difference (LSD) test at the 5% level of significance.

for Mn. Nutrients Ni and Zn did not show a clear trend between G and Y lines, but Ni content was higher in DES 119 (G), DP 5690 (G), DP 5690 (Y), MD 51ne (G), MD 51ne (Y), SG 747 (G) than the rest of the Y lines [bearing in mind that there are differences in Ni content among these lines; for example] [highest levels of Ni content was recorded in DP 5690 (Y) and MD 51ne (G)] [next was MD 51ne (Y)] [and last set has DES 119 (G)] [DP 5690 (G)] [and SG 747 (G)]. Zinc content was higher in DES 119 (G), DES 119 (Y), MD 51ne (G), SG 747 (G), and SG 747 (Y) than the rest of Y lines. Generally, with few exceptions, the content of C, N, P, B, Cu, Fe, and Mn was higher in G lines than their counterpart Y lines, especially in 2015. The nutrient content in seed (%) in 2015 ranged from 21.47 to 27.27 for protein (27% difference); 24.40 to 31.70 for oil (30% difference); 50.63 to 57.40 for C (13% difference); 2.50 to 4.47 for N (40% difference); 0.37 to 0.49 for S (32% difference); 0.10 to 0.16 for Ca 60; 1.04 to 1.2 for K (15% difference); 0.51 to 0.82 for P (37% difference); and (mg·kg^{-1}) 9.14 to 11.90 for B (30% difference); 42.77 to 64.07 for Fe (50% difference); 8.27 to 11.63 for Cu (41% difference); 10.80 to 15.17 for Mn (40% difference); 2.13 to 4.03 for Ni (88% difference); and 33.23 to 47.27 for Zn (42% difference).

3.3. Correlations between Nutrients

Correlation between nutrients in 2014 showed that there was no correlation between protein and nutrients and between oil and nutrients (Table 4). There were positive correlations between Ca with P, B, Cu, Fe, Mn, and Zn. Potassium positively correlated with B. The following positive correlations were also found: between P and C, N, B, Cu, Fe, Mn, and Ni; between C and Ni; between N and B, Cu, Fe, Mn, and Ni; between S and Fe and Zn; between B and Fe and Mn; between Cu and Fe, Mn, Ni, Zn; between Fe and Mn and Zn; between Mn and Ni and Zn. The pattern of correlation in 2015 was different from those of 2014. For example, both protein and oil positively correlated with P, C, N, B, Cu, Fe, and Mn in 2015 (Table 5), but this did not occur in 2014, where no correlation occurred between protein or oil with nutrients. Protein and oil were positively correlated. Oil was positively correlated with K. There was a positive correlation between Ca and C in 2015. Potassium positively correlated with B in 2014.

Phosphorus positively correlated with C, N, B, Cu, Fe, and Mn in 2015, but also correlated with Mn in 2014. Carbon was positively correlated with N, B, Cu, Fe, and Mn in 2015, differing from those of 2014 where positive correlation only occurred with Ni. Nitrogen correlation with nutrients in 2015 was similar to those in 2014, except for Ni where the correlation occurred in 2014. A positive correlation occurred between S and Ni in 2015, differing from those occurred between S and Fe and Zn in 2014. Boron positively correlated with Fe and Mn in 2014 and 2015, but was also correlated with Cu in 2015. Copper positively correlated with Fe only in 2015, differing from those in 2014 where Cu was positively correlated with Fe, Mn, Ni, and Zn. Iron positively correlated with Mn and Zn, similar to those in 2014; and Mn positively correlated with Zn, similar to that in 2014, but different in that Mn positively correlated with Ni in 2014.

Table 4. Pearson Correlation Coefficients (*P* and *R* values) between seed nutrients in four sets of near-isogenic cotton lines expressing green or yellow leaf color and their virescent parent grown in Stoneville, MS in 2014.

		Protein	Oil	Ca	K	P	C	N	S	B	Cu	Fe	Mn	Ni
Oil	R	0.262												
	P	0.186												
Ca	R	0.119	−0.108											
	P	0.554	0.591											
K	R	0.118	0.307	0.342										
	P	0.559	0.119	0.081										
P	R	−0.185	0.003	0.402	0.366									
	P	0.355	0.987	0.037	0.069									
C	R	−0.215	0.095	−0.209	0.161	0.493								
	P	0.281	0.636	0.296	0.423	0.009								
N	R	−0.038	0.112	0.217	0.314	0.554	0.257							
	P	0.851	0.578	0.278	0.111	0.003	0.195							
S	R	−0.132	0.051	0.164	0.098	0.068	−0.174	0.255						
	P	0.519	0.805	0.424	0.634	0.743	0.397	0.209						
B	R	0.139	0.232	0.446	0.418	0.519	−0.057	0.454	0.376					
	P	0.490	0.244	0.020	0.030	0.006	0.779	0.018	0.058					
Cu	R	−0.058	−0.047	0.411	0.245	0.559	−0.038	0.538	0.133	0.345				
	P	0.773	0.817	0.033	0.219	0.002	0.852	0.004	0.518	0.078				
Fe	R	−0.253	0.070	0.503	0.179	0.573	−0.079	0.427	0.404	0.412	0.741			
	P	0.202	0.729	0.008	0.371	0.002	0.695	0.026	0.041	0.033	<0.0001			
Mn	R	−0.059	0.117	0.705	0.374	0.752	0.173	0.553	0.108	0.567	0.569	0.731		
	P	0.769	0.562	<0.0001	0.055	<0.0001	0.390	0.003	0.599	0.002	0.002	<0.0001		
Ni	R	0.016	0.162	0.193	0.035	0.667	0.445	0.460	−0.367	0.099	0.420	0.262	0.58	
	P	0.935	0.419	0.335	0.863	<0.0001	0.020	0.016	0.066	0.622	0.029	0.187	0.002	
Zn	R	−0.057	−0.207	0.570	0.343	0.130	−0.346	0.186	0.514	0.349	0.480	0.632	0.446	−0.258
	P	0.776	0.301	0.002	0.080	0.517	0.077	0.354	0.007	0.074	0.011	<0.0001	0.020	0.194

Table 5. Pearson Correlation Coefficients (*P* and *R* values) between seed nutrients in four sets of near-isogenic cotton lines expressing green or yellow leaf color and their virescent parent grown in Stoneville, MS in 2015.

		Protein	Oil	Ca	K	P	C	N	S	B	Cu	Fe	Mn	Ni
Oil	R	0.632												
	P	0.0004												
Ca	R	0.254	0.083											
	P	0.202	0.680											
K	R	0.203	0.467	−0.126										
	P	0.311	0.014	0.532										

Continued

P	R	0.669	0.760	0.219	0.343									
	P	<0.0001	<0.0001	0.273	0.080									
C	R	0.650	0.642	0.397	0.332	0.758								
	P	<0.0001	<0.0001	0.040	0.090	<0.0001								
N	R	0.655	0.675	0.071	0.226	0.637	0.727							
	P	<0.0001	<0.0001	0.724	0.258	<0.0001	<0.0001							
S	R	−0.019	−0.036	−0.204	0.039	−0.361	−0.153	−0.153						
	P	0.924	0.860	0.308	0.846	0.065	0.445	0.448						
B	R	0.696	0.593	0.333	0.120	0.566	0.808	0.696	−0.005					
	P	<0.0001	0.001	0.090	0.550	0.002	<0.0001	<0.0001	0.979					
Cu	R	0.559	0.647	0.142	0.243	0.691	0.757	0.543	−0.238	0.678				
	P	0.002	<0.0001	0.480	0.222	<0.0001	<0.0001	0.003	0.232	<0.0001				
Fe	R	0.447	0.458	0.290	0.136	0.668	0.657	0.453	−0.211	0.415	0.373			
	P	0.019	0.016	0.142	0.500	<0.0001	<0.0001	0.018	0.290	0.031	0.056			
Mn	R	0.569	0.611	0.307	0.143	0.680	0.524	0.580	−0.195	0.506	0.293	0.546		
	P	0.002	0.001	0.119	0.475	<0.0001	0.005	0.002	0.331	0.007	0.138	0.003		
Ni	R	−0.114	0.056	−0.080	0.270	−0.023	0.256	0.045	0.449	0.148	0.193	−0.106	−0.228	
	P	0.572	0.780	0.692	0.173	0.910	0.198	0.823	0.019	0.461	0.335	0.599	0.254	
Zn	R	0.168	−0.060	0.321	−0.169	0.315	0.341	0.233	−0.218	0.202	−0.067	0.680	0.448	−0.107
	P	0.403	0.766	0.103	0.401	0.110	0.082	0.243	0.276	0.313	0.741	<0.0001	0.019	0.597

4. Discussion

The non-significant effect of line or year by line interaction for protein and K indicated that year and line by year interaction did not change the trend and response of these constituents, and the trend and response of these nutrients were similar in each year. This was the opposite for N, C, P, B, Cu, Fe, Mn where these constituents were significantly influenced by year, line, and their interaction, and their responses to the environmental conditions in each year were significant. The different responses of these constituents in each year could be due to the different environmental factors such as temperature (Figure 1) and the sensitivity of nutrients to temperature as the experiment was irrigated. It is worth to notice that although some nutrients such as oil and Ca were affected by year and line, they were not significantly influenced by their interaction, and this may reflect the level of sensitivity and stability of these constituents with the environmental conditions.

The higher oil content in some G lines in 2014 and in almost all G lines in 2015 than the Y lines indicated that the G lines showed a higher ability to accumulate more oil in seeds, and this may be due to higher photosynthesis capacity and higher rates of C and N metabolism, leading to higher oil synthesis. This explanation can be supported by a higher content of seed C and N in G lines than

in Y lines, especially in 2015 where growth conditions were more favorable for some nutrient uptake, assimilation, and protein and oil production. In addition, most nutrient levels, especially in 2015, were higher in G lines than in Y lines. These nutrients, including N, P, K, C, P, B, Cu, Fe, and Zn, were previously shown to be related to protein and oil in cotton and other species. For example, others studied the effects of K fertilization (0.0 and 57.1 kg of $K_2O \cdot ha^{-1}$), foliar Zn application (0.0 and 60 $mg \cdot l^{-1}$ Zn), and P (0, 600, 1200 and 1800 $mg \cdot l^{-1}$ of P_2O_5) on cottonseed yield and seed nutrition [52]. They found that the application of K, Zn, and P increased cottonseed yield, seed oil content, and seed oil and protein production (oil and protein·ha^{-1}). In recent research [35] where these NIL sets were compared with the obsolete parent SA30 for growth rates, yields, and fiber quality, it was found that incorporating the $v_1 v_1$ allele in modern lines led to higher yield in modern lines in spite of their yellow leaf color trait, indicating that these modern lines were bred for higher yields. This was supported by the fact that the boll yields and the plot weight measurements in yellow NILs was essentially a 2-fold increase in yield in the yellow NILs derived from the SA30. For example, bolls/plant were: compared with SA 30 (5.87 bolls per plant), the yellow modern lines had: DES 119 (G) (14.50) and DES 119 (Y) (13.27); DP 5690 (G) (12.52) and DP 5690 (Y) 14.73); MD 51ne (G) (13.37) and MD 51ne (Y) (11.82); SG 747 (G) (14.21) and SG 747 (Y) (13.52). Detailed yield and growth will be published elsewhere [35]. It was suggested that further molecular/biochemical characterization and comparisons of transcriptomes of the SA30 and the NILs research may explain cellular mechanisms and identify gene(s) controlling the increased reproductive structure yields [35]. The similar pattern between SA 30 and yellow modern lines was found for lint weight (data not shown).

The role of K, Zn, and P in CO_2 fixation, photosynthesis, carbon movement, electron transfer and energy production, and protein and carbon-related enzymes has been shown by others [52]-[56]. The role of P in photosynthesis process, carbon assimilation transport, and protein and oil has been previously shown [56] [57] [58]. Although our experiment did not suffer nutrient deficiency as soil had adequate soil nutrients, maintaining high content of these nutrients in leaves and seed would enhance seed quality traits, including protein and oil. Also, the role of Mg, S, and B were previously reported by others, who indicated that the application of $MgSO_4$ (1%) at 60 days after seedling (DAS) with combination of B at 0.1% at 75 DAS resulted in higher number of flowers per plant, number of bolls per plant, average boll weight, seed cotton yield and seed yield [59]. It was suggested that the positive effects of Mg, S, and B were due to the increased metabolic activities, protein synthesis, and mobilization of photosynthates [60] [61]. In addition, most of micronutrients such as Zn, Fe, Mn, Cu, B, and Mg are catalysts in enzymatic reactions, required for respiration, meristematic development, chlorophyll formation, photosynthesis, protein and oil synthesis, and gossypol, tannin and phenolic metabolism [58] [61] [62] [63]. The

information on the effects of phenotype trait such as leaf color on cottonseed nutrition using near-isogenic lines is limited. Previous research, conducted in our laboratory, [34] studied the effects of fuzzy/linted and fuzzless/linted seed phenotypes on cottonseed protein, oil, and minerals in near-isogenic *Gossypium hirsutum* cotton lines under field conditions. They found that protein content was higher in fuzzy genotype lines, but oil was higher in fuzzless genotype lines as the relationship between protein and oil is inversely correlated [34]. In addition, it was found that Ca and C content were higher in fuzzless genotypes than fuzzy, but N, S, B, Fe, and Zn content were higher in fuzzy than in fuzzless lines [34]. They concluded that seed fiber involved with N and C metabolism, affecting protein and oil composition and the mobility of nutrients from leaves (source) to seed (sink). A greenhouse experiment, conducted on similar lines under irrigated and non-irrigated conditions, showed similar results and achieved similar conclusions [33].

The significant differences between G lines and Y lines and between lines for nutrient content in seed could be due to environmental conditions of each year (E), genotype (G) differences, and G × E interactions. Some lines showed wide range of nutrient content compared with others. The response of nutrients in each year was different. Researchers screened 20 cotton genotypes for protein and oil and found that the frequency distribution showed normal distribution, and differences in protein and oil were significantly different among genotypes [64]. In addition, they reported that the relationship between protein and oil was inverse and complex. They suggested that these complex relationships are associated with genetics. Others analyzed the diversity of cottonseed protein and oil, and other seed quality components in the U.S. National Cotton Germplasm Collection [65]. They reported studies from nine genomes (one tetraploid and eight diploid), 33 species (five tetraploid and 28 diploid), and surveyed 2256 accessions. They found that oil content ranged from 8% to 27% and protein ranged from 10% to 36%, and reported that most of the range values were present within *G. hirsutum* and *G. barbadense*, suggesting that the variability of cottonseed protein and oil can be introduced into current breeding programs from these two species. They reported that there has been only slight variability in oil and protein content of new cotton cultivars over the past 19 years as found by the High-Quality Regional Test category of the National Cotton Variety Tests. For example, the first 5-year average was higher (20.2% oil; 22.1% protein) than the most recent 5 years (19.7% oil; 21.4% protein), indicating a decline. The slight decline and minor variation could be due to environmental effects and not genetics [66] [67]. The information here is useful for a breeding program to identify germplasm that can be used to select cotton varieties with high protein or oil content in cottonseeds.

A three-year experiment was conducted to investigate the genetic variability of seed oil content in 20 wild species, two cultivated species, and six perennial races of *Gossypium arboreum* cotton [68]. They found the mean seed oil content va-

ried from 10.26% to 22.89% in 22 species of *Gossypium* and six races of *G. arboreum*, and the highest level of oil was recorded in *G. lobatum* followed by *G. harknessii*. Because they found that five wild species of *Gossypium* surpassed the best lines and *G. arboreum* for seed oil, they suggested crosses between lines of *G. arboreum* and wild species of *Gossypium* can be promising. They concluded that such information is beneficial for efficient breeding selection for new cotton genotypes with improved cottonseed oil with a broad genetic base. A study was conducted on genetic variability, heritability, genetic gain and correlations for cottonseed soil in eight *G. hirsutum* cultivars and found significant differences among the genotypes for seed oil, which ranged from 27.52% to 30.15%, and a negative correlation was found between oil and yield [69]. Others evaluated oil content in 22 F_6 cotton lines (*G. hirsutum*) and found that there is genetic variability, and the overall mean oil content ranged between 23.52% and 24.51%, and they are higher than those of the Brazilian cultivar BRS Aroeira that have oil content of 22.04% [70]. They also repeated that the analysis of variance in each environment showed differences between the oil contents of the 22 lines. For example, in three locations the variation in oil content between the lines ranged from 21.67% to 27.59%, 20.1% to 24.2%, and 17.0% to 22.6%, respectively. However, the variation between checks in the three locations was smaller; ranged from 22.93% to 25.93%, 20.4% to 21.3%, and 19.4% to 19.8%, respectively. They indicated that a wide range of variability between lines, and between lines and checks, and between environments may be due to G × E interactions [70]. Our research showed wide averages; for example, the averages across two years ranged from 24% to 27% for protein and 10% to 30% for oil, and similarly for nutrients such as N, and minerals such as P, K, B, Fe, and Ni. It was concluded that the complex relationships between seed quality components and their variation, independently and in relation to each other, will provide a better tool to select parents for breeding programs for higher seed nutritional qualities [65]. They also reported that the development of cotton cultivars with genetic diversity and wide range of protein and oil will allow for basic studies in genetics, nutritional, and technological implications of protein and oil and their quality as cottonseed is a source of protein, oil, and minerals for human as food and livestock as feed. Growing the world population to nine billion by 2050 [71] will need efficient food production and maintaining high seed nutritional qualities.

Mapping and gene identification for the virescent gene *v*1 has been recently found. In an experiment conducted on mapping and identification of the virescent gene *v*1 in Upland cotton (*Gossypium hirsutum*), it was reported that young leaves of virescent mutants are yellow and gradually turn green as the plants reach maturity [37]. Mapping studies revealed a recessive gene (*v*1) from a virescent mutant of Upland cotton was found and narrowed to an 84.1-Kb region containing ten candidate genes. The *v*1 gene from the *v*1 mutant was mapped to chromosome D10, *Gh_D*10*G*0283/*GhChlI*, and is related to Chl synthesis and likely the cause of the gene for the virescent trait. The *v*1 gene

(GhChlI) encodes the cotton Mg-chelatase I subunit (CHLI) and was identified as the candidate gene for the virescent mutation. The accumulation of a chlorophyll biosynthetic intermediate, Mg-protoporphyrin (one of the plastid signals affecting the transcription of nuclear genes encoding chloroplast proteins [72]. The *GhChlI* gene has two copies, *Gh_A*10*G*0282 and *Gh_D*10*G*0283*,* and the coding region of *GhChlI* is 1269 bp in length, with three predicted exons and one non-synonymous nucleotide mutation (G1082A) in the third exon of *Gh_D*10*G*0283, with an amino acid substitution of arginine to lysine. *GhChlI*-silenced TM-1 plants exhibited a lower *GhChlI* expression level, a lower chlorophyll content, and the virescent phenotype. Analysis of upstream regulatory elements and expression levels of *GhChlI* showed that the expression of *GhChlI* could be normal, and with the development of the true leaf, the increase in the *Gh_A*10*G*0282 could partially for the deficiency of *Gh_D*10*G*0283 in the *v*1 mutant. Also, it was reported that although the protein sequence encoded by the third exon of *GhChlI* is highly conserved across diverse plant species, an amino acid substitution in conserved residues mainly resulted in leaf color changes in various species. Based on this, it was suggested that the mutation (G1082A) in the *GhChlI* gene may lead to a functional defect of the GhCHLI subunit and consequently to the virescent phenotype in the *v*1 mutant. Therefore, studying the *v*1 gene will increase further understanding of the genetic basis of virescent mutants and the regulatory mechanisms associated with chloroplast development and chlorophyll biosynthesis; the *GhChlI* mutation provides a tool to advance our understanding of the association between CHLI protein function and the chlorophyll biosynthesis pathway and impacts breeding programs [37]. In our research, modern yellow lines had higher lint yield than absolute yellow lines, indicating modern yellow lines contains in their genetic makeup some additional markers for higher-yielding trait; if this observation is exploited, it should advance our knowledge in developing higher-yielding cotton across other genotypes. Also, it was reported that leaf-color markers can be used to remove off-type plants in plant breeding [6]. This is because leaf-color mutations often result in abnormal chloroplast development and chlorophyll biosynthesis. This was supported by the results obtained by others [37] that the total chlorophyll content of the second leaf from the top of T582 was significantly lower (50% lower than the wild type parent, TM-1). The similar observation was noticed for chloroplast number per cell and lamellar structures where the number was significantly lower in T582 compared with that of the wild type, concluding that the *v*1 gene mutation disrupts the formation of thylakoids in the chloroplast, leading to chlorophyll accumulation [37].

It can be concluded that leaf-color mutants are effective tools to study chloroplast development and chlorophyl biosynthesis [73]; understanding the molecular mechanisms between mutations within GhChlI gene and GhCHLI protein function; linkage between the catalytic efficiency of Mg-chelatase and mutations within the *GhChlI* gene; linkage and between the mutation (G1082A) and the

interaction among the CHLI, CHLH, and CHLD subunits [37]; and contribute to the application of leaf-color markers in cotton breeding [6] [37]

The involvement of the *v*1 gene in chloroplast development was also shown by others [73]. They worked on rice *virescent*-2 mutant (*v*2), a temperature-sensitive mutant, and showed that the mutant develops chlorotic leaves at temperature (20˚C), but almost had normal green leaves at temperature (30˚C). This mutation is suggested to be responsible for plastid encoded proteins involved in photosynthesis and plastid transcriptional regulation during chloroplast differentiation; they also found that the translation of the plastid transcripts encoding the plastid transcription/translation apparatus was blocked at an early stage of chloroplast differentiation. They reported that the accumulation of transcripts of nuclear encoded photosynthetic genes was significantly suppressed in the mutant at later stages of chloroplast differentiation, but transcripts of genes accumulated at higher levels at later stages, concluding that the growth stage of plastid translation machinery of chloroplast differentiation is important for triggering the induction of nuclear-encoded chloroplast proteins at later stages. The accumulation of a chlorophyll biosynthetic intermediate, Mg-protoporphyrin [72].

Recently, using map-based gene cloning method [37], an improved bulked segregant analysis method was conducted in virus-induced gene silencing strategy for gene mapping to further fine-map the *v*1 locus. The method was applied to a multiple recessive marker Texas 582 (T582 upland cotton. They identified unique genomic positions of mutant loci. They reported that the *v*1 locus was mapped and one gene, *GhCHLI,* encodes one of the subunits of Mg-chelatase, was differentially down-regulated in T582 compared with TM-1. A point mutation occurred in the AAA+ conserved region of *GhCHLI* and led to an amino acid substitution. Suppression of its expression by virus-induced gene silencing strategy in TM-1 led to a yellow leaf phenotype. They reported that they identified the gene underlying the *v*1 locus encoding the CHLI subunit of Mg-chelatase I. Mg-chelatase I contains CHLI, CHLD and CHLH subunits in dicot plants. They further reported that the three subunits, CHLI and CHLD, have a similar ATPase structure and an amino acid (AAA+ domain in the N-terminus).

The positive correlations between nutrients in 2014 and 2015, especially between C, N, P, and other nutrients including B, Fe, Cu, and Zn were also previously reported by others on cotton and other species [38] [74]-[78]. The correlation between protein and nutrients and oil and nutrients that occurred in 2015 and not in 2014 could be due to the temperature differences between 2015 and 2014 as temperatures in 2015 were higher than in 2014 during the critical growing period (June-September). For example, the maximum temperatures in 2014 were 31.39˚C, 31.15˚C, and 32.49˚C, and 31.28˚C, respectively in June, July, August, and September (Figure 1). The temperatures in 2015 were 32.63˚C, 34.10˚C, 33.35˚C, 33.0˚C, respectively in June, July, August, and September (Figure 1) (Mississippi State Extension 2018). It was found that temperature or
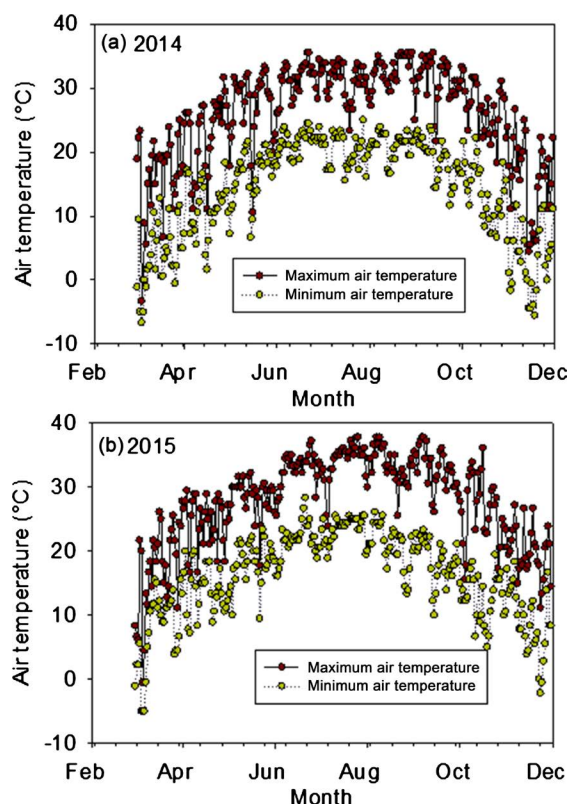
Figure 1. Air temperature (˚C) during the growing season in 2014 (a) and 2015 (b). The experiment was conducted in 2014 and 2015 in Stoneville MS. Source: Mississippi State University Extension (2018); http://extension.msstate.edu/agriculture/crops/cotton.

temperature differences between years can affect the accumulation and correlation between seed composition components in different species [38] [75] [76] [77] [78]. This could be due to temperature effects on the uptake rate of nutrients from the soil, and the movement of nutrients from leaves (source) to seed (sink) [33] [34]. They concluded that high temperatures during the growing season, and especially during the critical stages such as seed-fill, can alter seed composition. The inverse relationship between protein and oil was due to the genetically inherited trait between protein and oil [78]. The distribution of nutrients accumulation in seed in genotypes (**Figure 2** and **Figure 3**) showed uniform and bimodal distribution an confirm the efficiency differences of genotypes in accumulating nutrients as previously reported [33].

5. Conclusions

The current research demonstrated that a phenotype trait such as leaf color can influence seed protein, oil, and mineral nutrition as the leaf color may be involved in photosynthesis and N and C metabolism. It appears that green leaf isogenic lines accumulate more nutrients than the yellow color lines, and this was clearly and consistently observed in 2015. Temperature differences between the growing seasons can affect the relationships between protein and oil, and between protein and oil and other nutrients. In our case, year of 2015 was warmer
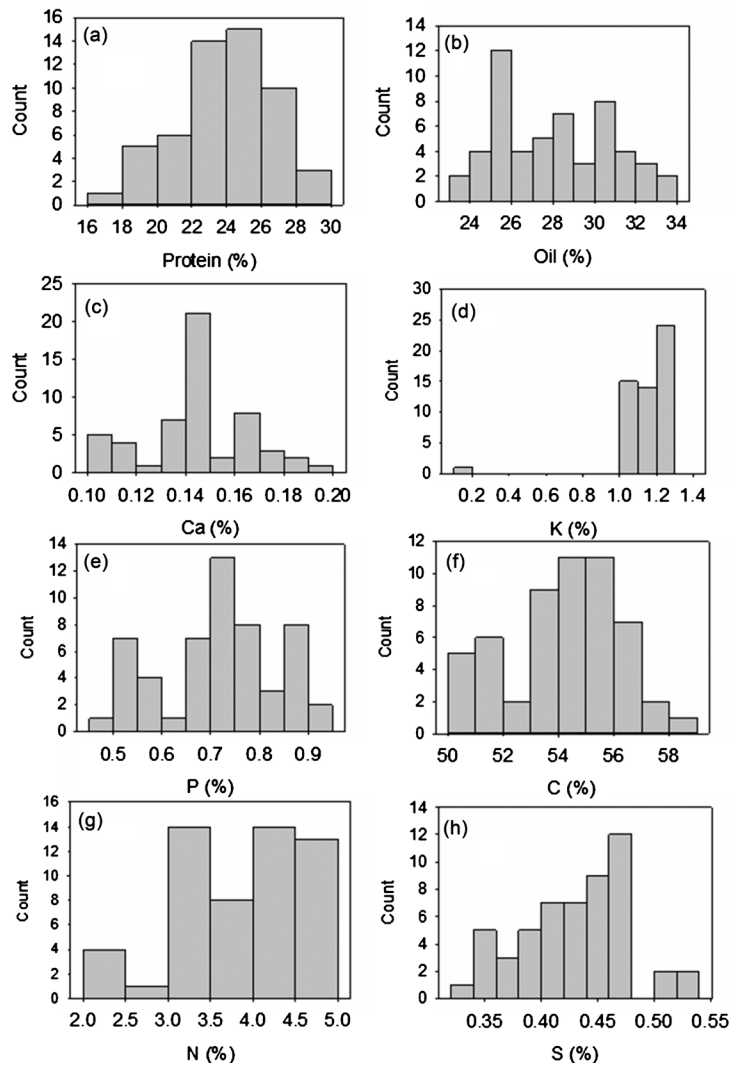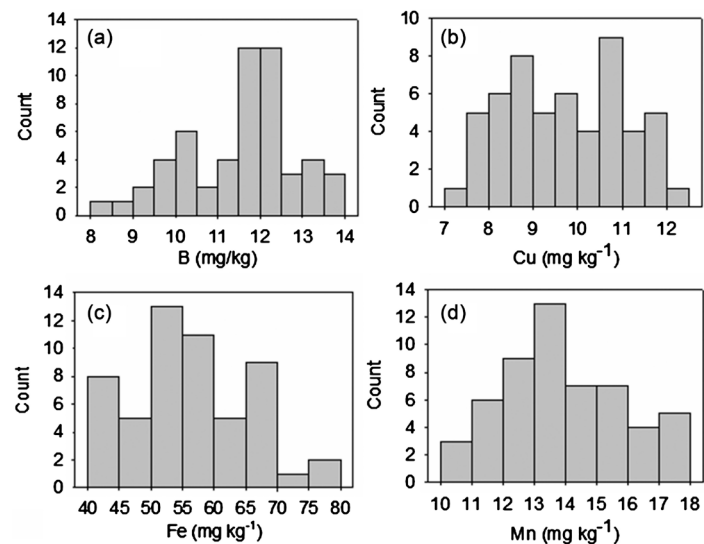
Figure 2. Distributions of seed protein, oil, Ca, K, P, C, N, and S (%) ((a)-(h)) across years and lines in near-isogenic cotton lines expressing green or yellow leaf color and the virescent parent. The experiment was conducted in Stoneville, MS in 2014 and 2015.
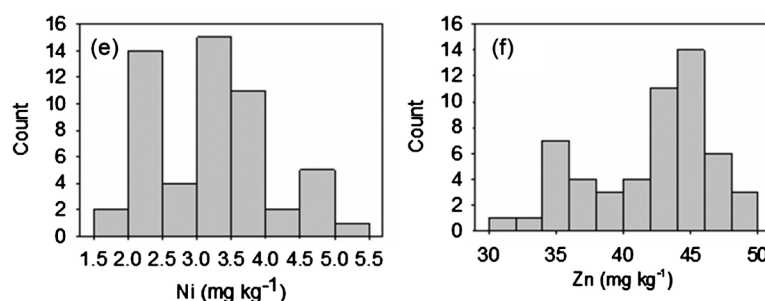
Figure 3. Distributions of seed B, Cu, Fe, Mn, Ni, and Zn (mg·kg^{-1}) ((a)-(f)) across years and lines in near-isogenic cotton lines expressing green or yellow leaf color and the virescent parent. The experiment was conducted in Stoneville, MS in 2014 and 2015.

than 2014, and this led to the fact that the correlation among minerals and between protein and minerals and between oil and minerals was different. The correlation between protein and nutrients and between oil and nutrients was different in each year, depending on the temperature of each year, reflecting the significant influence of environment effects on nutrient accumulation on the seed. The information in this research will benefit breeders in that both phenotype traits such as leaf color and environmental factors such as heat can affect the relationships between nutrients, including seed composition and mineral nutrition. The wide range of seed protein, oil, and minerals in these lines, allowing for opportunities for breeders to select for higher nutritional qualities in seeds. Further research is needed to investigate the effects of leaf color on seed amino acids and carbohydrates in these near-isogenic lines.

Acknowledgements

Author Contributions Statement

NB contributed to the experimental design, analysis of seed composition and mineral nutrition, interpretation, and manuscript writing. RBT contributed to the planning, design, data interpretation, and writing. SRS contributed to the planning, design, data interpretation, and writing. WTM contributed to the physiology aspect of the project.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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