Evaluation of Seed Protein Content in USDA Cowpea Germplasm

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Abstract. Cowpea [*Vigna unguiculata* (L.) Walp] is an annual legume crop grown worldwide to provide protein for human consumption and animal feed. The objective of this research was to evaluate the seed protein content in U.S. Department of Agriculture (USDA) cowpea germplasm for use in cowpea breeding programs. A field experiment was conducted with a randomized complete block design (RCBD) with three duplications in two locations, Fayetteville and Alma, in Arkansas, United States. A total of 173 USDA cowpea accessions were evaluated with the Elementar Rapid N analyzer III for their seed protein contents. The results showed that there was a wide range of seed protein content among the 173 cowpea genotypes, ranging from 22.8% to 28.9% with an average of 25.6%. The broad-sense heritability for seed protein among the 173 cowpea genotypes was 50.8%, indicating that seed protein content was inheritable and can be selected in breeding processing. The top five cowpea accessions with the highest seed protein contents were USDA accession PI 662992 originally collected from Florida (28.9%), PI 601085 from Minnesota (28.5%), and PI 255765 and PI 255774 from Nigeria and PI 666253 from Arkansas (28.4% each). PI 339587 from South Africa had the lowest protein content with 21.8%. There were also significant differences in seed protein contents observed among different seedcoat colors; the accessions with cream color exhibited higher protein content (27.2%) than others. This research could provide information for breeders to develop cowpea cultivars with higher seed protein content in a cowpea breeding program.

Cowpea [*Vigna unguiculata* (L.) Walp], an annual legume native to tropical and subtropical regions, is a protein-rich crop that complements staple cereal for human and fodder for livestock and also provides soil improvement benefits through nitrogen fixation. Cowpeas are commonly grown in the semiarid tropics between 35°N to 30°S of the equator, covering Africa, Asia, Oceania, the Middle East, Southern Europe, Central and South America, and the southern United States. The African countries of Nigeria and Niger account for 66% of world production. It is estimated that cowpeas are cultivated on 12.56 million hectares and have a worldwide

production of 5.55 million tons; they are consumed by 200 million people on daily basis in the 20 countries with highest cowpea cultivation (Boukar et al., 2018; Singh et al., 1997).

Cowpea can be used at all stages of growth (Fang et al., 2007; Nielsen et al., 1997). The green seeds can be used fresh or canned or frozen for humans. The young leaves, pods, and peas contain vitamins and minerals, which are used for human consumption and animal feed. Cowpea can be consumed as dry seeds, canned or frozen food (Fery, 1993), and as milling flour in baked goods (Kushwaha and Kumar, 2014). The seeds are also used for human consumption as an affordable source of protein and a supplemental fodder for livestock. In addition, cowpea has been used as an alternative to soybean for people who are allergic to soybean protein (Boukar et al., 2018).

Like soybean, cowpea is nutritious with ≈23% protein in dry seeds, which could meet the increasing consumer demand for healthier and more nutritious food. Unlike soybean, cowpea proteins do not cause allergies and are of higher quality when substituted in diets at equivalent protein contents. In recent years, there has been increasing interest in breeding cowpea cultivars with high seed protein content to improve nutritional quality. Evaluation of seed protein content in cowpea germplasm will help plant breeders select and breed high seed protein content cultivars in breeding programs. Asante et al. (2006) reported that protein content averaged 27.3% among 32 accessions. Gupta et al. (2010) screened 21 cowpea genotypes and observed that total protein content ranged from 22.4% to 27.9%; they found that seven genotypes (viz. HC-6, HC-5, CP-21, LST-II-C-12, CP-16, COVU702, and HC-98-64) had the highest protein contents, ranging from 26.7% to 27.9%. Itatit et al. (2013) studied 11 cowpea genotypes and reported a range of 20.57% to 24.95%. Afiukwa et al. (2013) found a greater variability of the total seed protein contents, ranging from 15.06% to 38.5%, with a mean of 25.99% in dry seeds among 110 cowpea genotypes. Oke et al. (2015) analyzed five varieties of cowpea and found that seed protein contents ranged from 25.80% to 28.95%. Ravelombola et al. (2016) assessed 11 cowpea cultivars/breeding lines developed in Arkansas and found an average protein content of 25.4% (range 23.7% to 27.4%) with a standard deviation of 1.9%. Weng et al. (2017) compared two methods to measure seed protein content and found a large variance among 240 cowpea genotypes.

Germplasm provides the elite gene(s) for breeding program. The objective of this study was to evaluate seed protein content among 173 worldwide cowpea accessions to use the high seed protein cowpea germplasm in cowpea breeding programs to develop superior cowpea cultivars with high seed protein contents.

Materials and Methods

Plant materials and field experiment. This study included 173 cowpea genotypes. The seeds were obtained from the USDA Germplasm Resources Information Network (GRIN) and increased in the field of University of Arkansas Research and Extension Center at Fayetteville, AR. The field experiments were conducted using a RCBD with three replications in two locations in Arkansas: Fayetteville (36°4'N, 94°9'S) and Alma (35°29'N, 94°13'S) (Supplemental Table 1). Each cowpea accession was planted in a single, 14-foot-long row, and the distance between rows (row spacing) was 3 feet. Plant spacing within rows was ≈4 inches. During the growing season, no pesticides, herbicides, or chemicals were sprayed to control pests, disease, or weeds. No irrigation was regularly maintained before maturity. The cowpea pods were bulk harvested when 90% of

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Pods were dried based on differing maturity. The cowpea seeds were shelled and cleaned after pods were harvested.

Seed sample preparation. A total of 1038 samples were collected from the 173 cowpea accessions at the two locations, with three duplications at each location. Before measuring seed protein content, each cowpea genotype was further selected for matured seeds, uniform color and size, and lack of damage from insects or machinery. To have a sufficient quantity of seeds for protein analysis, ~20 g of cowpea seeds from each sample were ground using a coffee grinder (Hamilton Beach) for 1 min. Approximately 5 g of the ground powder was sieved through 100# (nominal wire diameter 0.1 mm), and 1 g of each sample was weighed and then transferred to a 0.2-mL microfuge tube for protein determination by N/protein analyzer.

Seed protein content assessment. Cowpea seed protein content was measured by analyzing the percentage of Nitrogen by combustion using an Elementar Rapid N III instrument at Agriculture Diagnostic Laboratory, University of Arkansas. Each 1.0-g sample was collected and measured for protein content. At high temperature and in the presence of pure oxygen, nitrogen was removed by combustion. The nitrogen was then isolated from other combustion products. A thermal conductivity detector measured the nitrogen content for each sample (Horneck and Miller, 1998). The percentage of nitrogen in each sample was provided, and the total protein content for each sample was estimated by times 6.25 nitrogen (Moore et al., 2010).

Data analysis. Analysis of cowpea seed protein data were performed by analysis variance (ANOVA) using the general linear models procedure of JMP Genomics 7 (SAS Institute, Cary, NC). For comparisons among genotypes, Student's *t*-test was used to perform multiple comparisons for least square mean protein content at *P* = 0.05. The mean, range, SD, SE, and CV were estimated for seed protein content using Tabulate, and the distributions of protein content was also performed using Distribution in JMP Genomics 7.

On the basis of our experiments in this study, there were 173 cowpea genotypes growing in two locations using RCBD with three blocks (replications) in each location. ANOVA for seed protein contents among the 173 cowpea genotypes was conducted (Table 1) using the two-way genotype × environment (GE) model suggested by Singh et al. (1993). The broad-sense heritability (*H*²) was estimated using the formula.

$$H^2 = 100 * (\sigma_G^2 / \sigma_P^2) \\ = 100 * (\sigma_G^2 / (\sigma_G^2 + \sigma_{GE}^2 + \sigma_e^2)),$$

where σ_P^2 is phenotypic variance, σ_G^2 is genotypic variance, σ_{GE}^2 is genotype × environment variance, and σ_e^2 is variance associated with the experimental error. σ_G^2 , σ_{GE}^2 , and σ_e^2 were obtained using the following formula:

$$\sigma_G^2 = (MSG - MSGE) / bL,$$

$$\sigma_{GE}^2 = (MSGE - MSE) / b,$$

$$\sigma_e^2 = MSE,$$

where MSG is mean square genotype, MSGE is mean square G × E, and MSE is mean square error. The estimates of MSG, MSGE, and MSE were taken from the ANOVA table; *b* is the number of blocks (*b* = three blocks in this study), and *L* is the number of locations (*L* = 2 locations here) (Table 1).

Seed protein content-based seedcoat color In this study, based on the seedcoat color, there were 18 cowpea accessions with black seedcoat, 26 blackeye, 29 browneye, 12 cream, 8 pinkeye, 15 red, 19 red Holstein, 26 tan, and 20 varied (Table 3). ANOVA based on seedcoat and multiple comparisons using Student's *t*-test were also analyzed with JMP Genomics 7.

Results and Discussion

Elite germplasm discovery. Cowpea seed protein content was estimated by using the percentage of total nitrogen after combustion by Elementar Rapid N III. Significant variations were observed for the seed protein content among 173 USDA cowpea accessions (Fig. 1), ranging from 21.8% to 28.5% with an average of 25.6% (Supplemental Table 1; Fig. 1).

The results were in agreement with previous reports for cowpea seed protein content at the 25% level (Afiukwa et al., 2013; Asante et al., 2006; Boukar et al., 2018; Itatit et al., 2013; Nielsen et al., 1997; Oke et al., 2015; Singh et al., 1997; Ravelombola et al., 2016). The top 10 USDA cowpea accessions with higher protein contents (≥28%) are listed in Table 2. Among them, the USDA cowpea accession PI 662992 from Florida had the highest seed protein content with 28.9% of the dry seed weight. PI 601085 was second highest with 28.5%, collected from Minnesota. The two accessions PI 255765 and PI 255774 from Nigeria and PI 666253 from Arkansas had protein contents as high as 28.4%. PI339587 from South Africa had the lowest protein level (21.8%) (Table 2; Supplemental Ta-

ble 1). The five cowpea genotypes with high seed protein contents, PI 662992, PI 601085, PI 255765, PI 255774, and PI 666253, could be used in breeding programs aimed at developing varieties with high protein content.

The cowpea is an important food crop in tropical and subtropical regions, but little research has been done on seed protein content in this crop compared with the other legume species such as soybean (*Glycine max* L.). Due to limited data on germplasm with high seed protein content available to use in cowpea breeding program, it is necessary to screen cowpea germplasm to find elite genotype(s) with high protein content. Whether the cowpea seeds are used as food for direct human consumption or processed into flour to make baked goods or other products, the protein content of cowpea seeds is an important index closely related to quality, health, nutrition, and market price. Farmers and consumers prefer high-protein varieties and products. Therefore, high protein content has become a goal in cowpea breeding programs and production. Previous studies have confirmed that cowpea seeds in general had a 25% protein content. It will be valuable to find varieties and germplasm with protein content greater than the average of 25%. Asante et al. (2006) studied the variation in protein content of 32 cowpea accessions in Ghana and found the seed protein content ranged from 16.4% to 27.3% with an average of 22.5%. Two among the 32 accessions had seed protein content higher than 25%. 'Bengpla (1)' had the highest with 27.3%, and another breeding line, 87/30, had 26.9% seed protein content (Asante et al., 2006). Gupta et al. (2010) screened cowpea for total seed protein content in 21 genotypes and found that the seed protein contents ranged from 22.4% to 27.9% and reported that 'HC-98-64' had the highest protein content at 27.9%. Ravelombola et al. (2016) evaluated 11 released cowpea cultivars and breeding lines developed in Arkansas and found that the average protein content was 25.4% (range 23.7% to 27.4%). Cultivar Early Scarlet and the breeding line 09-204 had the highest and the second highest seed protein content with 27.4% and 26.9% dry seed weight, respectively (Ravelombola et al., 2016). In the current study, more than 10 elite USDA cowpea accessions were found to have high seed protein content of greater than 28.4%, which is higher than regular commercial cowpea cultivar(s) (Table 1; Supplemental Table 1); these could be used in cowpea breeding program to develop new cultivar(s) with higher protein content. Our study provides

Table 1. Analysis of variance for seed protein contents among 173 cowpea genotypes.

Source	df	Sum of squares	Mean square	F	Prob > F	EMS	MS symbol	Variance
Environment (E)	L - 1 = 1				Not relevant			
Blocks/Env	(b - 1)L = 4				Not relevant			
Genotype (G)	v - 1 = 172	2429.31	14.12	14.74	<0.0001	$\sigma_e^2 + b\sigma_{GE}^2 + bL\sigma_G^2$	MSG	$\sigma_G^2 = 1.80$
G × E	(v - 1)(L - 1) = 172	566.76	3.30	3.44	<0.0001	$\sigma_e^2 + b\sigma_{GE}^2$	MSGE	$\sigma_{GE}^2 = 0.78$
Error	(v - 1)(b - 1)L = 688	659.09	0.96			σ_e^2	MSE	$\sigma_e^2 = 0.96$

EMS = estimated mean square; G × E = gene × environment; MSG, mean square genotype; MSGE, mean square gene × environment.

valuable information for breeders to select and use those germplasms to develop new cowpea cultivars.

Low protein content germplasm was also found in USDA germplasm accessions such as PI 221730, PI 292890, PI 339587, PI 582671, PI 582819, and PI 582850 with protein content from 21.8% to 22.7%, and all except PI 582819 (Supplemental Table 1) had red Holstein seedcoats.

ANOVA showed that there were significant differences among the cowpea genotypes and the interaction of genotype $\times$ environment (Table 1), suggesting a significant genotype effect and genotype $\times$ environment effect. As shown in Table 1, MSE was 0.96; MSGE was estimated to be 3.30; and MSG was estimated to be 14.12. Broad-sense heritability H^2 for cowpea seed protein among the 173 cowpea genotypes was $\approx 50.8\%$, indicating the seed protein content was highly inheritable. Ajeigbe et al. (2008) reported that a broad-sense heritability in cowpea was 86% based on

nine cowpea varieties. Nielsen et al. (1993) had a high broad-sense heritability of 95% for seed protein in 100 cowpea lines based on data from one location without replication of the study. Tchiagam et al. (2011) determined a broad-sense heritability of 74% for seed protein using five divergent lines through 5×5 half diallel cross mating. Emebiri (1991) reported the broad-sense heritability for protein content ranging from 70% to 78% in two crosses. In our previous study, the broad-sense heritability was identified as 57.8% for seed protein content based on 11 Arkansas cowpea lines. All these studies indicate that cowpea has high heritability of seed protein content, suggesting that it is possible to select plants and lines with high seed protein for cowpea breeding programs. The results also show that estimation of the broad-sense heritability dramatically varied among cowpea genotypes from different experiments and studies. In both our studies, we found lower seed protein contents (50% to 60%) than reports from others ($>70\%$) due to a high interaction of genotype $\times$ environment observed in our experiments, which decreased the estimated heritability values.

Effects of environment on protein content.

In this study, two experimental locations exhibited similar cowpea seed protein content. The mean seed protein content of 173 cowpea accessions in Alma and Fayetteville, AK, were 25.67% and 25.58%, respectively, a nonsignificant difference between the two locations ($P = 0.05$). The University of Arkansas campus farm in Fayetteville ($36^\circ 4'N$, $94^\circ 9'S$) is on the outskirts of the Boston Mountains, deep within the Ozarks. The Vegetable Research Station in Alma

($35^\circ 29'N$, $94^\circ 13'S$) is located in the Arkansas River Valley at the edge of the Ozark Mountains, 50 miles away from the Fayetteville location. There is little difference in weather, temperature, and soil conditions between the two locations. Both are zone 7a according to the USDA Plant Hardiness Zone Map. The short distance and different conditions between the two locations did not affect seed protein content in cowpea. Different ecological regions and geographic environments should be considered if cowpea protein content is evaluated in future research.

The seed protein content in 173 USDA cowpea germplasm were similar in two experimental locations, with no significant difference was observed between locations. Some germplasm accessions were virtually the same at the two testing sites. For example, there was little difference between PI 250416 and PI 663151 in seed protein content (0.002% and 0.010%, respectively) at the two experimental locations. However, there were large differences in some accessions between the two sites. For example, the seed protein content of accession PI 663148 was 26.9% in Alma and 20.3% in Fayetteville, respectively, showing a clear difference between the two locations. Similarly, PI 583513 had 28.3% in Alma and 23.1% in Fayetteville (Supplemental Table 1).

Interaction between germplasm and location. ANOVA in this study showed that genotype and the genotype $\times$ environment interaction significantly influenced protein content ($P < 0.0001$) (Table 2). However, environmental effect by location was not significantly different due to the close geographic locations of the two sites, which have similar growing environments ($P = 0.1196$). A previous study by Bliss et al. (1973) indicated that there was a significant genotype $\times$ location interaction effect on cowpea protein content. Ddamulira and Santos (2015) also found that protein content in cowpea seeds was significantly affected by the interaction between genotype and environment. Research by Ravelombola et al. (2016) indicated that protein content was significantly different among cowpea genotypes and environment. The data in our study were similar to previous reports indicating that the genotype and interaction between genotype and environment contribute to seed protein content in cowpea. Significant differences in protein content and the interaction

Fig. 1. Distribution of seed protein contents among 173 U.S. Department of Agriculture cowpea germplasm.

Table 2. Top 10 U.S. Department of Agriculture cowpea germplasm accessions with highest seed protein content.

Accession	Protein content (%)	Seedcoat color	Origin
PI662992	28.9	Cream	Florida, USA
PI601085	28.5	Browneye	Minnesota, USA
PI255765	28.4	Blackeye	Nigeria
PI666253	28.4	Cream	Arkansas, USA
PI255774	28.4	Browneye	Nigeria
PI582942	28.3	Cream	Puerto Rico
PI430687	28.2	Red	China
PI666251	28.1	Browneye	Virginia, USA
PI664519	28.1	Cream	South Carolina, USA
PI666269	28.0	Pinkeye	Alabama, USA
PI250416	28.0	Cream	Pakistan
PI666262	28.0	Pinkeye	Georgia, USA
PI339587 (CK)	21.8	Red Holstein	South Africa

Table 3. Seed protein content parameters by seedcoat color.

Seed color	N	Mean	SD	Min.	Max.	Variance	SE	CV
Black	18	25.86 b ²	1.35	23.22	29.69	1.83	0.13	5.23
Blackeye	26	25.96 b	1.77	21.85	29.47	3.12	0.13	6.81
Browneye	29	25.96 b	1.77	21.85	29.47	3.12	0.13	6.81
Cream	12	27.23 a	1.59	20.26	30.19	2.53	0.19	5.85
Pinkeye	8	26.71 a	1.55	23.50	31.01	2.39	0.21	5.79
Red	15	25.25 c	1.69	21.39	29.28	2.87	0.18	6.71
Red Holstein	19	23.69 d	1.60	19.63	29.23	2.57	0.15	6.77
Tan	26	25.32 c	1.67	19.98	30.86	2.78	0.13	6.59
Varied	20	25.61 bc	1.40	22.01	29.11	1.95	0.13	5.46
Merged	173	25.63	1.82	19.63	31.01	3.31	0.06	7.10

²Significant difference at $P = 0.05$ level.

between genotype and environment were also observed in the 173 cowpea genotypes in our study (Table 2), indicating a strong genotype $\times$ environment interaction, although environment (location) effect was not significant.

Relationship between seed protein content and seedcoat color. Seedcoat color varies greatly in cowpea (Mann, 1914) and can be an important indicator of protein content among farmers growing cowpeas and for human consumption. Seedcoat color is not only an important agricultural trait to distinguish different cultivars and genotypes but also a steady indicator of seed nutrition quality. Fery (1985) noted that determining the mechanism of seedcoat pigmentation is complex and difficult to ascertain. Seedcoat color in cowpea is a complex trait, and there may be many genes involved color inheritance (Boukar et al., 2018; Egbadzor et al., 2014). There were at least 12 seedcoat colors recorded in the USDA cowpea germplasm database GRIN. Our results show that there were significant differences among the nine groups of the cowpea seedcoat colors (Table 3). Cream color had the highest protein content among the 173 USDA cowpea accessions (27.23%), and pinkeye cowpea was the second highest (26.71%). The two highest protein content colors were not significantly different from each other, but they had a total protein content significantly higher than other color types. Brown eye, black eye, and black color were not significantly different from each other and thus represent the third highest accessions in protein content: 25.96%, 25.96%, and 25.86%, respectively. Red Holstein color had the lowest seed protein content with 23.61% dry seed weight, which was significantly lower than other color coats (Table 3). Although cowpea seed protein content cannot be measured by seedcoat color in the marketplace, seedcoat color could provide customers with an indicator of which cowpeas might have higher protein content than others. From this study, we observed the cowpea seeds with cream or pinkeye color contained higher protein contents than others while the red holstein color seed had the least protein content.

Conclusions

In this study, 173 USDA cowpea germplasm accessions were measured for their seed protein contents. A wide range of seed protein contents was observed, from 21.8% to 28.9%, with an average of 25.6%. The accessions with the top 10 highest seed protein contents ($\geq 28.4\%$) were originally collected from the United States, Nigeria, Puerto Rico, China, and Pakistan. The results indicate that both genotype and the genotype $\times$ environment interaction significantly influence seed protein

content in cowpea. However, the environmental effect (location) was not significant due to the close distance and similar growing regions of the two locations in this study. The broad-sense heritability was 50.8% for the cowpea seed protein content based on 173 cowpea genotypes, indicating that seed protein content was inheritable and could be selected for cowpea breeding. Seedcoat color was also observed to be related to protein content. Cream and pinkeye expressed higher protein contents than other cowpea seedcoat colors, and the red Holstein contained the lowest protein content. These data provide meaningful information for consumers to choose cowpea products with high seed protein content according to different seedcoat colors in the marketplace.

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Supplemental Table 1. Seed protein content in 173 cowpea germplasm accessions.

Accession	Seed color	Protein content (%)
PI291140	Black	25.2
PI292898	Black	26.1
PI339588	Black	25.4
PI349674	Black	24.4
PI354673	Black	26.1
PI354832	Black	27.9
PI354835	Black	26.5
PI354843	Black	26.1
PI354860	Black	26.6
PI354864	Black	24.8
PI354865	Black	25.5
PI427093	Black	27.9
PI582474	Black	25.4
PI582575	Black	27.1
PI583197	Black	27.4
PI583232	Black	24.2
PI610520	Black	25.8
PI610620	Black	24.5
PI201498	Blackeye	23.1
PI229734	Blackeye	26.0
PI253428	Blackeye	27.0
PI255765	Blackeye	28.4
PI262179	Blackeye	26.2
PI293477	Blackeye	26.1
PI293505	Blackeye	26.0
PI293586	Blackeye	26.7
PI293588	Blackeye	26.5
PI406285	Blackeye	26.0
PI582352	Blackeye	26.6
PI582353	Blackeye	25.8
PI582422	Blackeye	25.4
PI582428	Blackeye	27.5
PI582466	Blackeye	26.0
PI582551	Blackeye	25.0
PI582923	Blackeye	26.2
PI583200	Blackeye	24.5
PI583513	Blackeye	25.7
PI608035	Blackeye	24.3
PI610533	Blackeye	25.7
PI642160	Blackeye	26.1
PI663101	Blackeye	26.6
PI663148	Blackeye	23.6
PI664517	Blackeye	26.8
PI664524	Blackeye	26.9
PI218123	Browneye	24.9
PI220851	Browneye	23.0
PI223023	Browneye	24.0
PI255774	Browneye	28.4
PI293470	Browneye	24.8
PI312210	Browneye	25.6
PI339599	Browneye	27.9
PI406290	Browneye	26.1
PI582512	Browneye	27.1
PI582570	Browneye	25.9
PI582573	Browneye	27.7
PI582667	Browneye	25.0
PI582814	Browneye	24.9
PI582855	Browneye	25.5
PI582857	Browneye	25.8
PI582861	Browneye	27.5
PI582863	Browneye	25.3
PI582926	Browneye	26.9
PI582932	Browneye	24.4
PI583201	Browneye	27.3
PI583202	Browneye	23.1
PI583248	Browneye	26.1
PI583249	Browneye	26.2
PI583274	Browneye	24.1
PI601085	Browneye	28.5
PI601453	Browneye	26.2
PI663011	Browneye	23.9
PI663059	Browneye	27.8
PI666251	Browneye	28.1

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Supplemental Table 1. (Continued) Seed protein content in 173 cowpea germplasm accessions.

Accession	Seed color	Protein content (%)
PI250416	Cream	28.0
PI582924	Cream	27.3
PI582942	Cream	28.3
PI592369	Cream	24.5
PI592374	Cream	26.1
PI662992	Cream	28.9
PI663140	Cream	25.5
PI663151	Cream	27.1
PI664515	Cream	26.6
PI664518	Cream	26.7
PI664519	Cream	28.1
PI666253	Cream	28.4
PI582525	Pinkeye	24.9
PI582931	Pinkeye	26.8
PI663150	Pinkeye	26.6
PI664513	Pinkeye	26.2
PI664514	Pinkeye	26.8
PI666260	Pinkeye	27.3
PI666262	Pinkeye	28.0
PI666269	Pinkeye	28.0
PI152195	Red	23.4
PI152197	Red	25.4
PI293587	Red	24.9
PI311119	Red	25.6
PI339605	Red	23.0
PI354805	Red	24.1
PI354854	Red	24.3
PI354855	Red	25.2
PI430687	Red	28.2
PI578902	Red	25.6
PI578911	Red	25.1
PI582702	Red	27.5
PI582815	Red	26.9
PI582868	Red	23.5
PI583195	Red	25.9
PI202802	Red Holstein	24.7
PI220850	Red Holstein	24.0
PI221730	Red Holstein	22.1
PI221731	Red Holstein	23.5
PI257463	Red Holstein	23.8
PI292890	Red Holstein	22.5
PI292892	Red Holstein	23.1
PI339587	Red Holstein	21.8
PI339591	Red Holstein	23.4
PI582423	Red Holstein	23.2
PI582468	Red Holstein	27.6
PI582671	Red Holstein	22.7
PI582703	Red Holstein	23.5
PI582812	Red Holstein	23.4
PI582822	Red Holstein	23.5
PI582823	Red Holstein	25.1
PI582824	Red Holstein	23.1
PI582850	Red Holstein	22.2
PI582858	Red Holstein	23.7
PI180014	Tan	26.4
PI190191	Tan	24.7
PI292894	Tan	26.0
PI292899	Tan	27.0
PI292913	Tan	26.1
PI300173	Tan	25.2
PI354580	Tan	24.2
PI367921	Tan	26.3
PI527561	Tan	26.2
PI582402	Tan	26.2
PI582415	Tan	24.4
PI582425	Tan	24.3
PI582465	Tan	26.3
PI582572	Tan	26.4
PI582697	Tan	26.6
PI582735	Tan	23.4
PI582789	Tan	23.5
PI582819	Tan	22.7
PI582825	Tan	23.0

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Supplemental Table 1. (Continued) Seed protein content in 173 cowpea germplasm accessions.

Accession	Seed color	Protein content (%)
PI582852	Tan	25.5
PI582874	Tan	23.9
PI582894	Tan	24.7
PI583209	Tan	27.4
PI583240	Tan	27.3
PI583244	Tan	26.0
PI583550	Tan	24.8
PI152196	Variable	26.3
PI167284	Variable	23.7
PI227829	Variable	26.4
PI244571	Variable	25.7
PI292897	Variable	25.6
PI293476	Variable	26.1
PI293514	Variable	24.8
PI293545	Variable	24.7
PI293570	Variable	25.2
PI293582	Variable	23.3
PI293584	Variable	26.5
PI339610	Variable	24.6
PI582417	Variable	25.7
PI582420	Variable	26.2
PI582421	Variable	25.7
PI582521	Variable	25.5
PI582530	Variable	27.2
PI582821	Variable	24.9
PI582856	Variable	26.8
PI583199	Variable	27.3