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Comparison of Morphological Traits in Cowpea Pod Length, Seed Number per pod, Seed
Weight, Seed Color and Seed Density in USDA Germplasm Accessions and Arkansas Breeding
Lines

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Abstract

The objective of the study was to compare and correlate the Arkansas cowpea breeding lines to the USDA cowpea germplasm accessions on the following morphological traits: seed color, pod length, number of seeds per pod, 100 seed weight, and seed density. The seed density is defined as the weight of seed in gram per centimeter of pod length. Three hundred seventeen cowpea genotypes were used in this experiment. Out of the 317, 285 germplasm accessions were from the USDA GRIN and 32 lines were from the Arkansas breeding program.

T-test between Arkansas breeding lines and USDA germplasm accessions were done to compare the two. The Arkansas breeding lines had a higher 100 seed weight than the USDA germplasm accessions. However, the USDA germplasm accessions had a higher seed number per pod and seed density than the Arkansas breeding lines. The Arkansas breeding lines did not differ from the USDA germplasm in the pod length.

The correlation analysis among the five traits showed the seed number per pod was highly positively correlated with seed density, while the pod length was highly negatively correlated with 100 seed weight. The pod length was moderately positively correlated with 100 seed weight, while the seed density was moderately negatively correlated with 100 seed weight. The seed number per pod and 100 seed weight were not correlated. The seed number per pod and pod length were not correlated. Seed color was not correlated to any of the mentioned morphological traits.

From this study, several potential USDA germplasm accessions were found to have favorable morphological traits. PI666260 (29.00 g), PI592374 (25.3 g), and 09.481 (25.00 g) had high the seed weight; PI582417 (30.5 cm), PI578902 (26.1 cm) and PI427093 (26.0 cm) had long pod; PI352997, PI527561, PI367921, PI292898, PI527302, PI152195 (18 seeds per pod)

and PI582697 and PI291140 (19 seeds per pod) had more seed per pod; and PI582932 (1.25 g/cm), PI583202 (1.19 g/cm), and PI339603 (1.19 g/cm) had high seed density.

Introduction and Literature Review

Cowpea (*Vigna unguiculata* L. Walp) is a diploid legume species ($2n=22$). The center of origin has been questioned between Africa and India, but Africa is generally referred to as the center of origin. Most researchers contend that West Africa is the center of origin, but some researchers argue that Swaziland may be the primary center of origin because Swaziland had the higher species diversity throughout the world (Oyewale, 2013). Cowpea is referred to as black-eyed pea or southern pea in some literature. Cowpea plants grow well in dry condition with as rainfall as little as 300mm (Gomez, 2004).

Cowpea is produced all over the world. The main locations of production are Africa, United States of America., Brazil, India, and Southeast Asia. The Food and Agriculture Organization (FAO) estimates that 3.3 million tonnes of cowpea dry grain were produced worldwide in 2000 (Gomez, 2004). Sixty eight percent of the cowpea were grown in Africa where Nigeria produced the most cowpea followed by Brazil and Niger.

The cowpea is a multipurpose crop. Cowpea plants are often used as cover crop to control weed emergence or double cropped with other crops such as sorghum. The cowpea plant could be dried after harvest and be used as hay or used as green manure. The cowpea grains are the main product of the cowpea plant, but the other parts of the plant are usable as well. The cowpea grains are mainly used for human and animal consumption. The cowpea leaves are used as salads for humans and forage for animals. The cowpea roots can fix nitrogen from the soil and provide nutrition to the plant and subsequent crop. The cowpea plant is a fabulous plant that is versatile for various uses with zero waste.

Among the possible crops to choose to study, cowpea was chosen for various reasons. Cowpea is an important grain legume in the diet of many people in the third world countries as it

provides not only high-quality protein, approximately 26.6%, but also constitutes the cheapest source of dietary protein for low income sectors of the population. Cowpea is also a good source of approximately 56.24% carbohydrates, 26.61% protein, 3.99% lipid, 8.60% moisture, 3.84% ash, 1.38% crude fiber, 1.51% gross energy, 54.85% nitrogen-free extract, and additionally contains calcium, iron, vitamin B and carotene (Gomez, 2014; Pottorff, 2012). Legumes are a special crop due to the fact that the plant can fix their own nitrogen as soybeans do. Soybeans can fix around 109 kg N per hectare while cowpeas fix around 50 kg N per hectare (Martins, 2003). Soybean has more nutrients compared to cowpea. However, soybean is less tolerant to drought stress than cowpea. Today, water scarcity and water resource management are becoming a major issue in agricultural production. Cowpea could be the next major crop, replacing soybean one day. More research is needed for cowpea to be competitive in the near future.

Agricultural production is a career where preparing ahead is key to success. Choosing a cowpea cultivar to plant out of thousands of cultivars can be difficult. The University of Arkansas has an excellent breeding program for cowpea and has produced numerous breeding lines. The breeding lines are high yielding and are suited for the climate in Arkansas and other regions as well. However, the differences between the breeding line and other cultivars are not clear. In this project, seed color, pod length, number of seeds per pod, 100 seed weight, and seed density were collected. Many factors affect yield potential, but the traits above were selected. The pod length affects the number of available seeds. Generally, the longer the pod the more seeds per pod. Pod length was chosen because collecting the data on pod length is easy. The number of seeds per pod was chosen for similar reasons. One hundred seed weight was chosen because the cowpea seeds are reported in weight per area. For example, bushel per acre. The seed density was included because the density could be calculated by the 100 seed weight and

pod length. Seed color was chosen because many people are interested in eating certain colored seed. Traits such as number of pods per plant and harvest yield were not selected because the field was not sized to accommodate numerous replications.

The objectives of this study were to observe five seed and pod morphological traits including seed color, pod length, number of seeds per pod, 100 seed weight and seed density among various cowpea cultivars, and to compare the statistics on Arkansas cowpea lines with other cowpea genotypes from other sources and to identify superior Arkansas cowpea lines for cowpea production.

Materials and Methods

Plant Material

A total of 351 cowpea genotypes collected from 30 countries were used for this study (Table 1). The cowpea cultivars were planted at the Fayetteville Research and Extension Experimental Center, University of Arkansas, AR. The planting plot was set as 10 x 3 feet growing, 20 plants in a row-plot during 2018. Weed control, water and fertilizer applications were applied following standard management practices. Five morphological traits: seed color, 100-seed weight, pod length, seed number per pod, and seed density were observed.

Seed Color

The seed colors were categorized using examples from the seed collection we have. The seeds were categorized into one of the colors: Black, Blackeye, Black Holstein, Cream, Tan, Brown eye, Brown Holstein, Red, Red eye, Red Holstein, and Variable. The variable seed color

is a color that could not be categorized into the other colors. Few seeds could not be differentiated and was categorized as darkpinkeye and darkbrowneye.

Seed Weight

The seed weight was weighed using an Ohaus Scout Pro SP401. One hundred seeds were randomly sampled to be weighed with 3 replications. When 100 seeds were not available, 50 seeds were weighed and multiplied by 2 or 25 seeds were weighed and multiplied by 4 based on the available number of seeds.

Pod Length

The pod length was measured using a standard 12 inch ruler. Ten pods were randomly sampled. When the pods were curved, the pod was broken into straight pieces and measured.

Seed Number per Pod

The seed number per pod was calculated by counting the number of seed per pod from the randomly sampled pod. Two immature seeds were counted as one mature seed.

Seed Density

The seed density was calculated by dividing the seed weight by the pod length (g/cm of pod). The average 100 seed weight was divided by 100 to get the average weight of one individual seed. Afterward the weight was multiplied by the number of seed per pod and divided by the pod length. The seed density was replicated 10 times for each randomly sampled pod.

$$\text{seed density} = \frac{(\text{average weight of one seed (g)} * \text{number of seed per pod})}{\text{pod length (cm)}}$$

Statistical Analysis

The data of the five cowpea morphologic traits were analyzed using analysis of variance (ANOVA) with the general linear models (GLM) procedure of JMP Genomics 9 (SAS Institute, Cary, NC). Best linear unbiased predictions (BLUP) for each line were calculated using the

MIXED procedure where genotypes were considered random. The descriptive statistics were generated using ‘Tabulate’; the distribution of the data was drawn using ‘Distribution’; and Correlation between quantitative-based data was calculated using “Correlation” options of JMP Genomics 9 (SAS Institute, Cary, NC).

Results and Discussion

Overview

Three hundred and fifty one genotypes were used in this study. However, data for 34 of the cowpea accessions were not obtained and data for 317 cowpea accessions were collected. Some cowpea accessions were not available to harvest because of long maturity season needed or climate not suitable for the growth.

100-Seed Weight

The 100 seed weight (g) was measured among 317 cowpea accessions with 3 replications each. The 100-seed weight was approximately normally distributed (Fig 1) and varied from 6.03 g to 29.00 g, with an average of 13.63 g and a standard deviation of 3.89 g. The genotypes with the three highest 100-seed weight were PI666260 (29.00 g), PI592374 (25.3 g), and 09.481 (25.00 g). The lines with the lowest 100-seed weight were PI194210 and PI610604 (6.83 g), PI583247 (6.13 g) and PI218123 (6.03 g).

A t-test analysis was conducted using the JMP Genomics 9 (Fig 2). Two hundred and eighty five USDA germplasm accessions were compared to 32 AR breeding lines. The AR breeding lines were normally distributed and varied from 9.23 g to 25 g, with an average of 16.26 g and a standard deviation of 3.98 g. On the other hand, the USDA germplasm had a more kernel distribution and varied from 6.03 g to 29.00g, with an average of 13.33 g and a standard

deviation of 3.77 g. There was a statistically significant difference and the AR breeding lines had a higher 100 seed weight than the USDA germplasm accessions (p-value less than .05).

Pod Length

The pod length (cm) was measured among 317 cowpea accessions with 10 replications each. The pod length was approximately normally distributed (Fig 3) and varied from 11.14 cm to 30.5 cm, with an average length of 16.74 cm and a standard deviation of 2.83 cm. The genotypes with the longest pod length were PI582417 (30.5 cm), PI578902 (26.1 cm) and PI427093 (26.0 cm). The lines with the shortest pod length were PI221730 (11.6 cm), ErectSet (11.2) cm, and PI189374 (11.1 cm).

A t-test analysis was conducted using the JMP Genomics 9 (Fig 4). Two hundred and eighty five USDA germplasm accessions were compared to 32 AR breeding lines. The AR breeding lines were normally distributed and varied from 11.16 cm to 21.95 cm, with an average of 17.47 cm and a standard deviation of 2.58 cm. On the other hand, the USDA germplasm accessions were also normally distributed and varied from 11.14 cm to 30.5 cm, with an average of 16.66 cm and a standard deviation of 2.85 cm. There was no statistically significant difference between the means of the USDA germplasm accessions and the AR breeding lines (p-value more than 0.05).

Seed Number per Pod

The seed number per pod was measured among 317 cowpea accessions with 10 replications each. The seed number per pod was normally distributed (Fig 5) and varied from 7.3 to 18.7 seeds per pod, with an average number of 13.15 seeds per pod and a standard deviation of 2.17 seed per pod. The genotypes with the most seeds per pod were PI352997, PI527561, PI367921, PI292898, PI527302, PI152195 (18 seeds per pod) and PI582697 and PI291140 (19

seeds per pod). The lines with the least seeds per pod were PI582422, 09.481, PI582417, PI578911, EarlyScarlet (8 seeds per pod) and PI430687 (7 seeds per pod).

A t-test analysis compared the 285 USDA germplasm accessions to the 32 AR breeding lines (Fig 6). The AR breeding lines were approximately normally distributed with a range of 8 to 14.8 seeds per pod, an average of 11.47 seeds per pod with a standard deviation of 1.50. The USDA germplasm accessions were approximately normally distributed with a range of 7.3 to 18.7 seeds per pod, and an average of 13.34 and a standard deviation of 2.15. There was a statistically significant difference and the USDA germplasm accessions had more seed number per pod than the AR breeding lines (p-value less than 0.05).

Seed Density

The seed density was measured among 317 cowpea accessions with 10 replications each. The seed density was approximately normally distributed (Fig 7) and varied from 0.26 to 1.25 gram per centimeter of pod (g/cm of pod), with an average of 0.80 (g/cm of pod) and a standard deviation of 0.16. The lines with the highest density were PI582932 (1.25 g/cm of pod), PI583202 (g/cm of pod), and PI339603 (1.19 g/cm of pod). The lines with the lowest density were PI430687 (0.41 g/cm of pod), PI578911 (0.34 g/cm of pod), and PI582417 (0.26 g/cm of pod).

A t-test analysis compared the 285 USDA germplasm accessions to the 32 AR breeding lines (Fig 8). The AR breeding lines were approximately normally distributed with a range of .45 to 1.06 gram per centimeter of pod with an average of 0.67 (g/cm of pod) and a standard deviation of 0.14. The USDA germplasm accessions were left skewed and varied from a range of 0.26 to 1.25 gram per centimeter of pod, with an average of 0.82 (g/cm of pod) and a standard

deviation of 0.15. The difference was statistically significant and the USDA germplasm accessions had a higher seed density than the AR breeding lines (p-value less than 0.05).

Seed Color

The seed color was categorized into 14 different colors: black, black holstein, black eye, brown, brown holstein, brown darkeye, browneye, cream, dark pink eye, red, red holstein, tan, and variable. The most common seed colors were tan (18.9%), variable (13.3%) and brown eye (12.6%) (Fig 9). There was no significant difference between seed color and any of the morphological traits studied.

Correlation

There was a high positive correlation (0.631) between seed number per pod and seed density, while the pod length was negatively correlated (-0.615) with seed density. As the seed number per pod increased, so did the seed density. On the other hand, when the pod length went up, the seed density decreased. The pod length and 100 seed weight were moderately positively correlated (0.513), while the seed density and 100 seed weight were moderately negatively correlated (-0.589). As the pod length increased, so did the 100 seed weight. As the seed density increased, the 100 seed weight decreased. There was little to no correlation between seed number and pod length. There was little to no correlation between seed number per pod and pod length (Table 7).

There was no correlation between seed color and any of the morphological traits (p value above 0.05) (Tables 8, 9, 10, 11).

Conclusion and Future Study

In order to breed a better cowpea, breeding programs are needed to select genotypes with better genes. Five seed and pod morphological traits were observed in this study. Afterward, the USDA germplasm accessions and AR breeding lines were compared. The AR breeding lines had a higher 100 seed weight than the USDA germplasm accessions. However, the USDA germplasm accessions had a higher seed number per pod and seed density than the AR breeding line. The AR breeding lines and the USDA germplasm accession had no difference in pod length. The USDA germplasm accessions had a higher seed density than the AR breeding lines although the AR breeding lines had a higher 100 seed weight and no difference in pod length because of the number of seed per pod. For example, the AR breeding line had a seed weigh 0.16 g with 15 seeds per pod while the USDA germplasm accession had a seed weigh of 0.13 g and 19 seeds per pod. The AR breeding line ends with 240 g total and the USDA germplasm ends with 247 g total. The correlation analysis shows the relationship between the 3 traits of 100 seed weight, number of seeds per weight, and seed weight very well. A high positive correlation between seed number per pod and seed density. A high negative correlation between seed density and 100 seed weight. Little to no relation between 100 seed weight and seed number per pod. A trade off of having more seeds but little seeds or having less seeds but heavy big seeds.

From this study, the genotype PI661260 had the highest 100 seed weight with 29 g. The genotype PI582932 had the highest seed density with 1.25 g/cm of pod. The genotype PI582417 had the longest pod length with 30.5 cm. The AR breeding lines are focused in obtaining consistent high yield with high nutrition. Further studies will be needed before the genotypes are incorporated into the AR breeding lines.

For future studies, I will replicate the experiment two more times. Multiple replications will reduce the probabilities of error and make the results more valid and reliable. For the

genotype that scored the highest in each morphological trait, I will find the origin and more background information about why the genotype scored very high. In the next study, I will compare the statistics to the USDA Germplasm Resource Information Network (GRIN) and find the genotype that did better at the Fayetteville Research and Extension Experimental Center, University of Arkansas, AR.

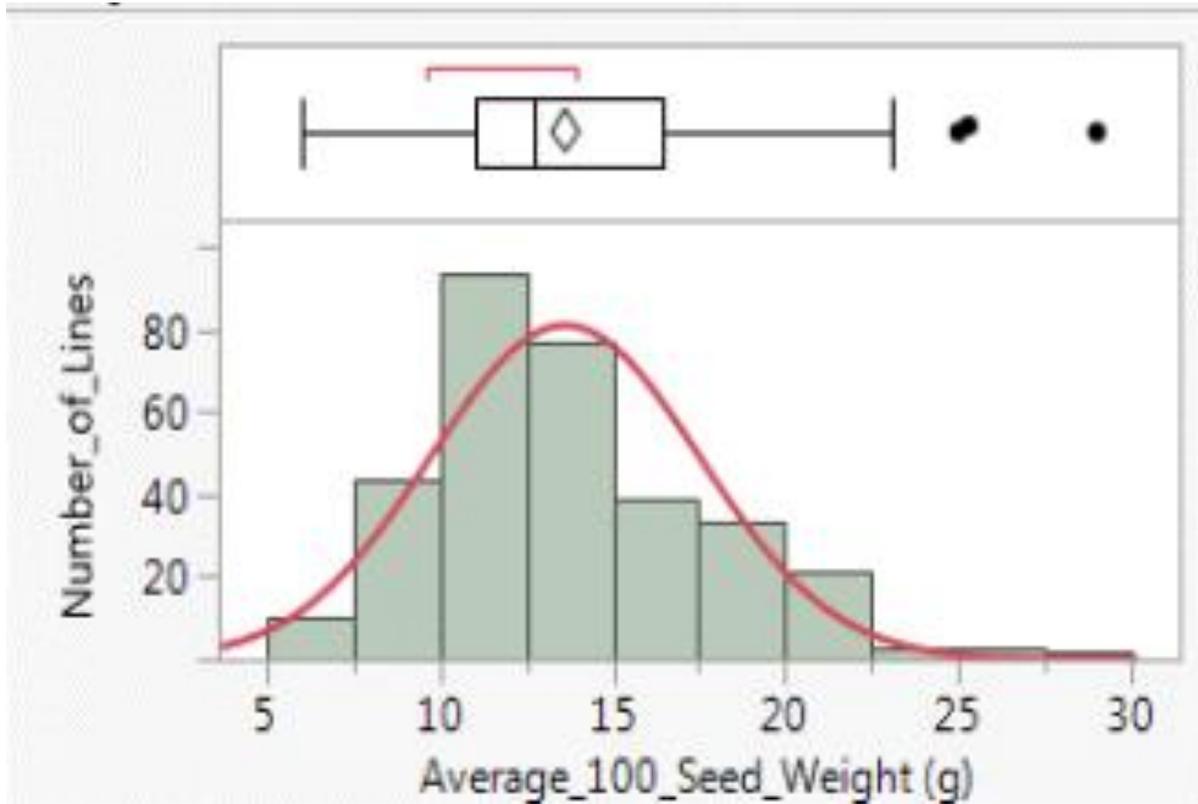
Acknowledgements

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doi:10.1186/1471-2164-13-234

Figures and Tables



Mean	Median	Max	Min	Std Dev	Std Err Mean	N
13.63 g	12.73 g	29 g	6.03 g	3.89	0.22	317

Fig1. Distribution of average 100 seed weight (g) among 317 cowpea accessions.

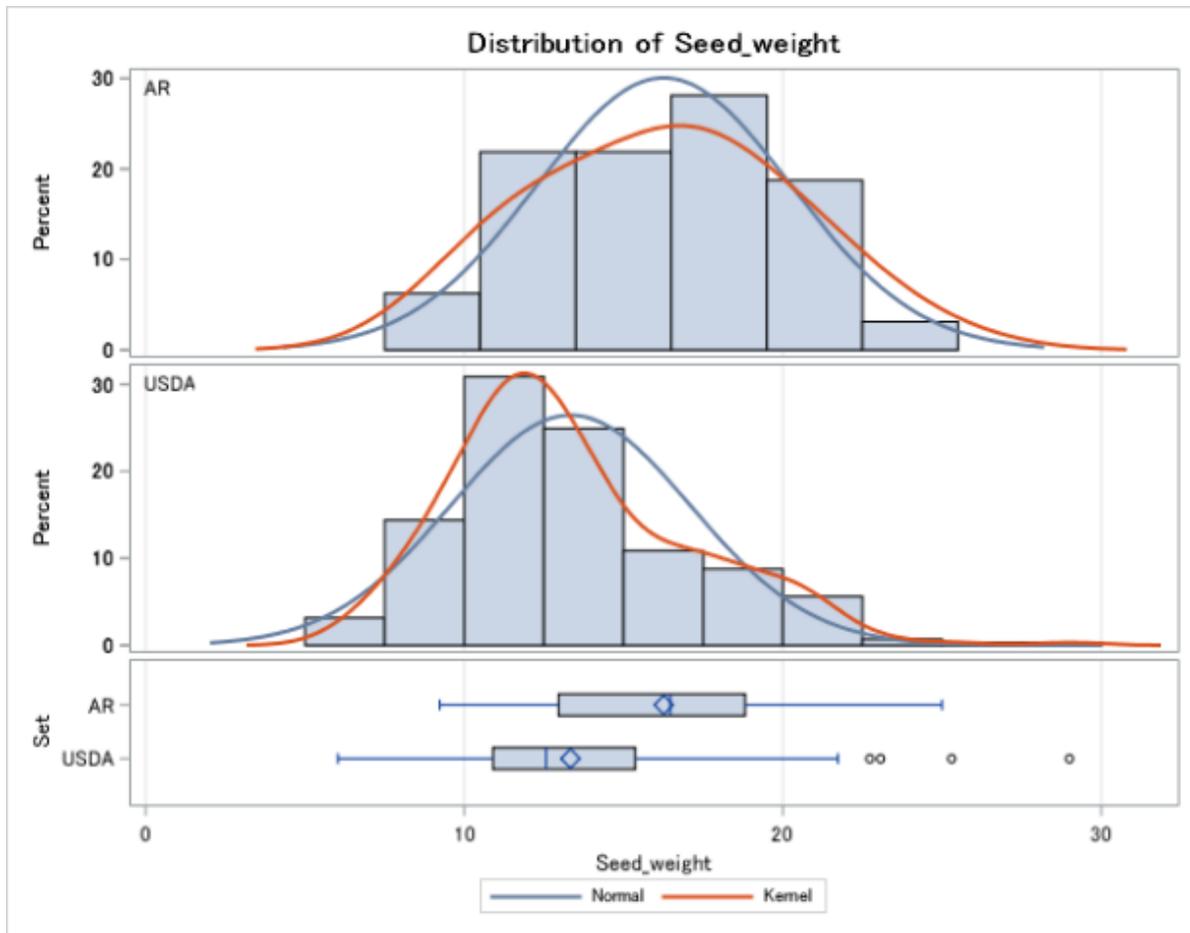
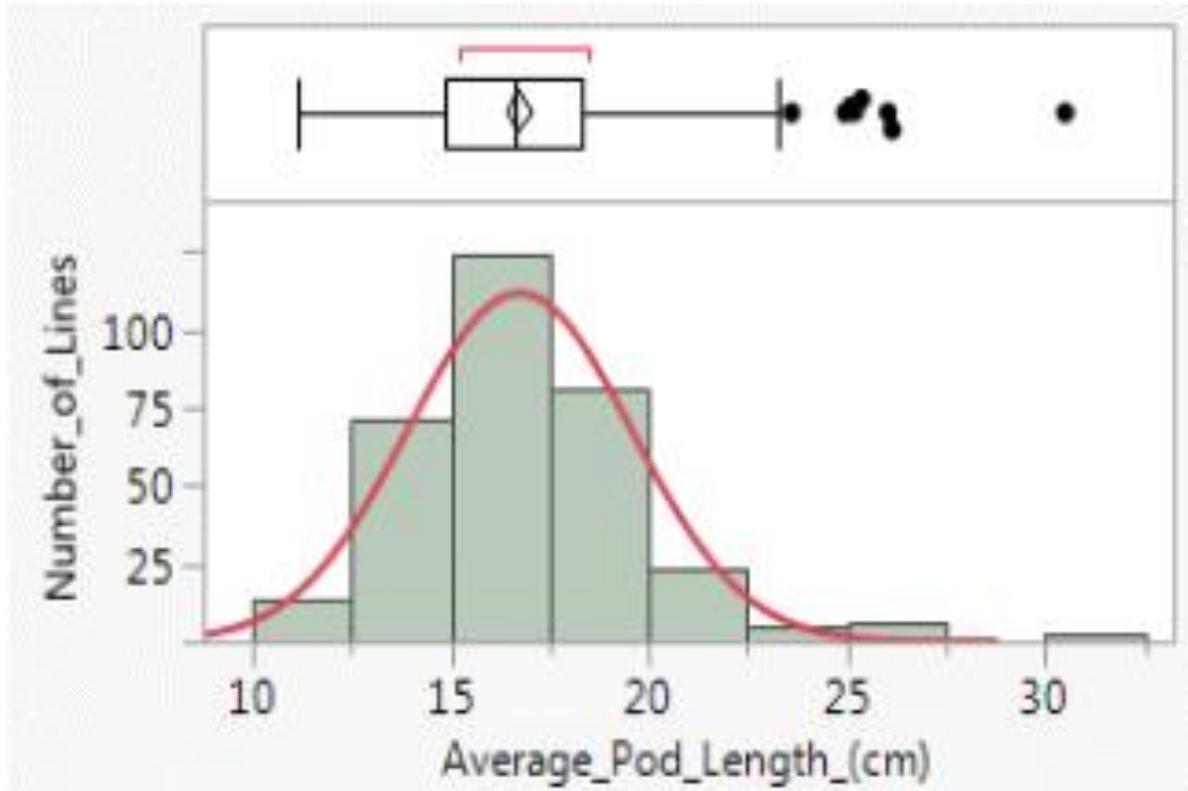


Fig 2. Distribution of AR breeding lines and the USDA germplasm 100 seed weight. AR breeding line has higher average 100 seed weight than USDA germplasm, and the difference is statistically significant.



Mean	Median	Max	Min	Std Dev	Std Err Mean	N
16.74 cm	16.65 cm	30.5 cm	11.14 cm	2.83	0.16	317

Fig3. Distribution of average pod length (cm) among 317 cowpea accessions

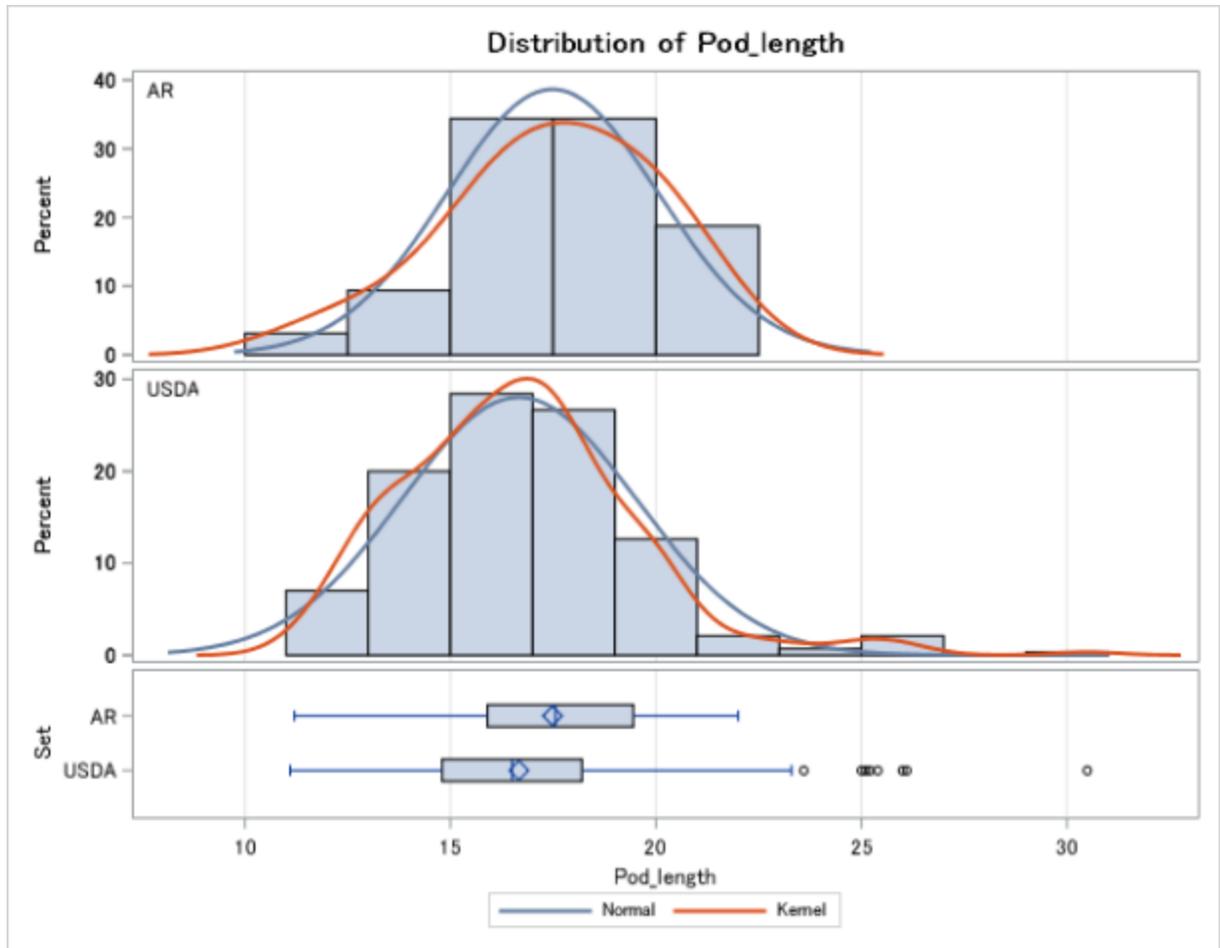
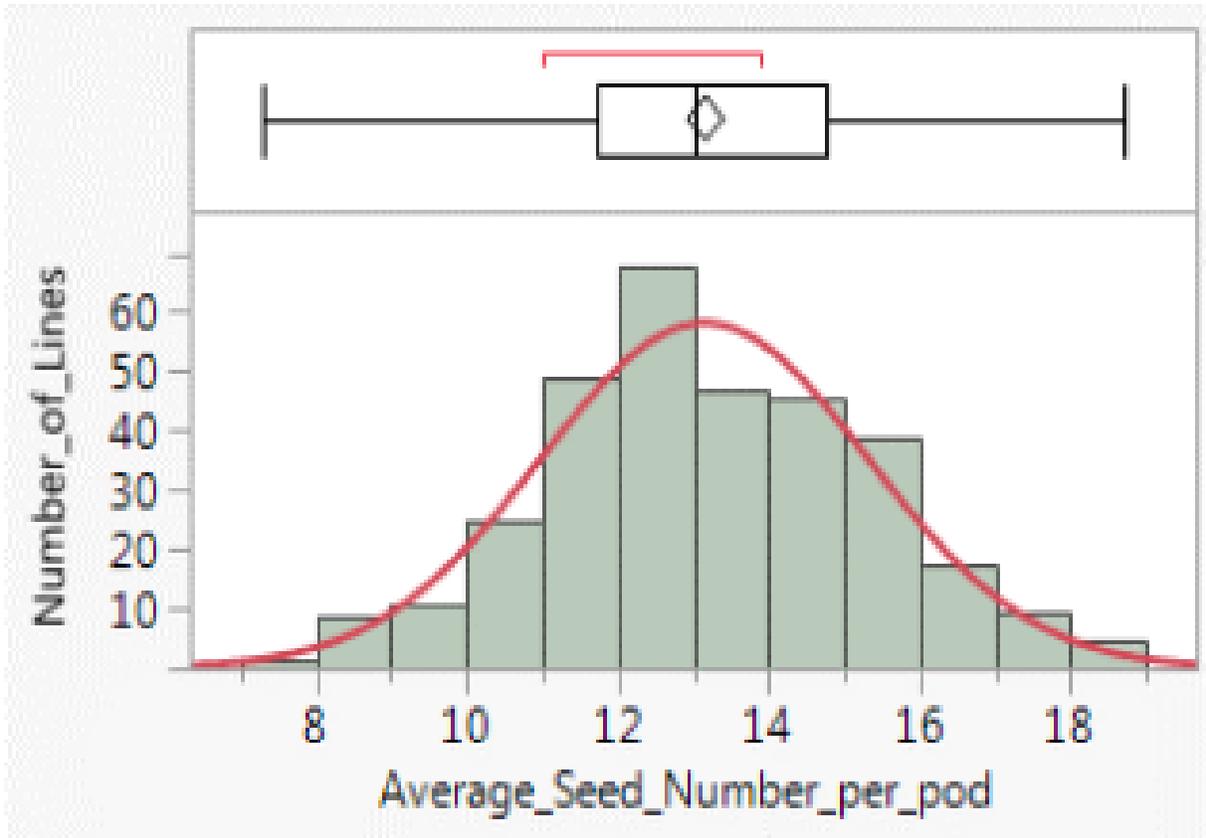


Figure 4. Distribution of pod length (cm) between AR breeding line and USDA germplasm. The AR breeding line has a slightly longer pod length (cm) than USDA germplasm, but the difference is not significant.



Mean	Median	Max	Min	Std Dev	Std Err Mean	N
13.15	13	18.7	7.3	2.17	0.12	317

Fig 5. Distribution of average seed number per pod among 317 cowpea accessions

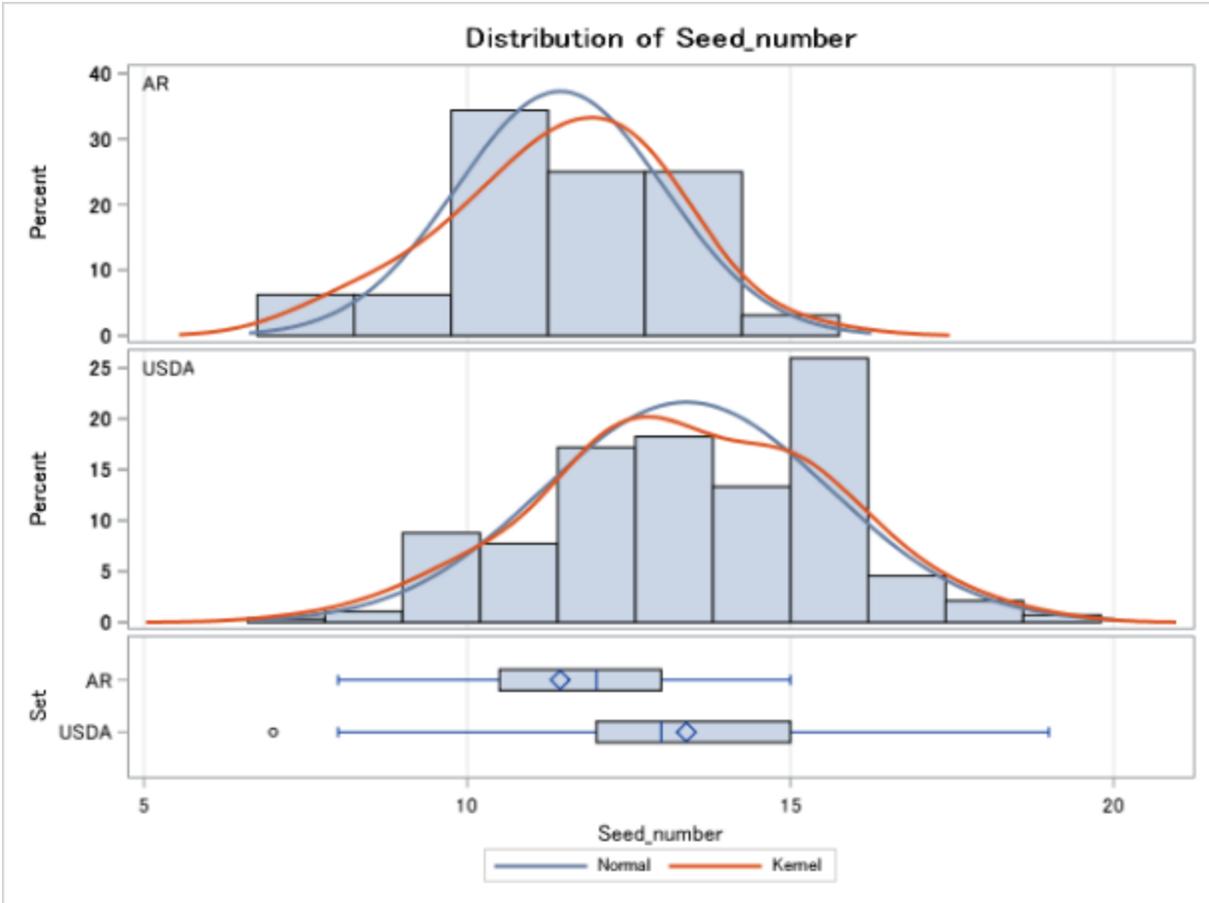
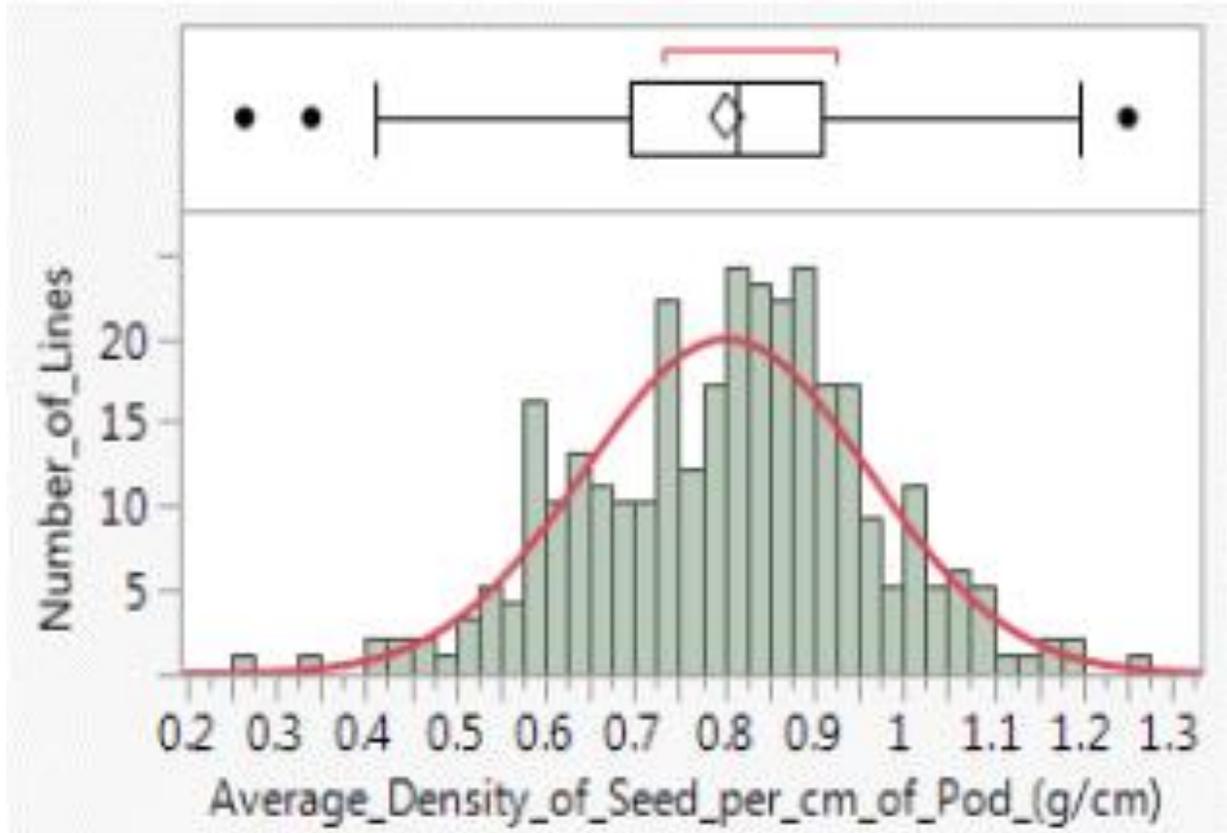


Figure 6. Seed number per pod between AR breeding line and USDA germplasm. The USDA germplasm has higher seed number per pod than the AR breeding lines and the difference is statistically significant.



Mean	Median	Max	Min	Std Dev	Std Err Mean	N
0.80 g/cm of pod	0.81 g/cm of pod	1.25 g/cm of pod	0.26 g/cm of pod	0.16	0.01	317

Fig 7. Distribution of average density of seed per cm (g/cm) among 317 cowpea accessions

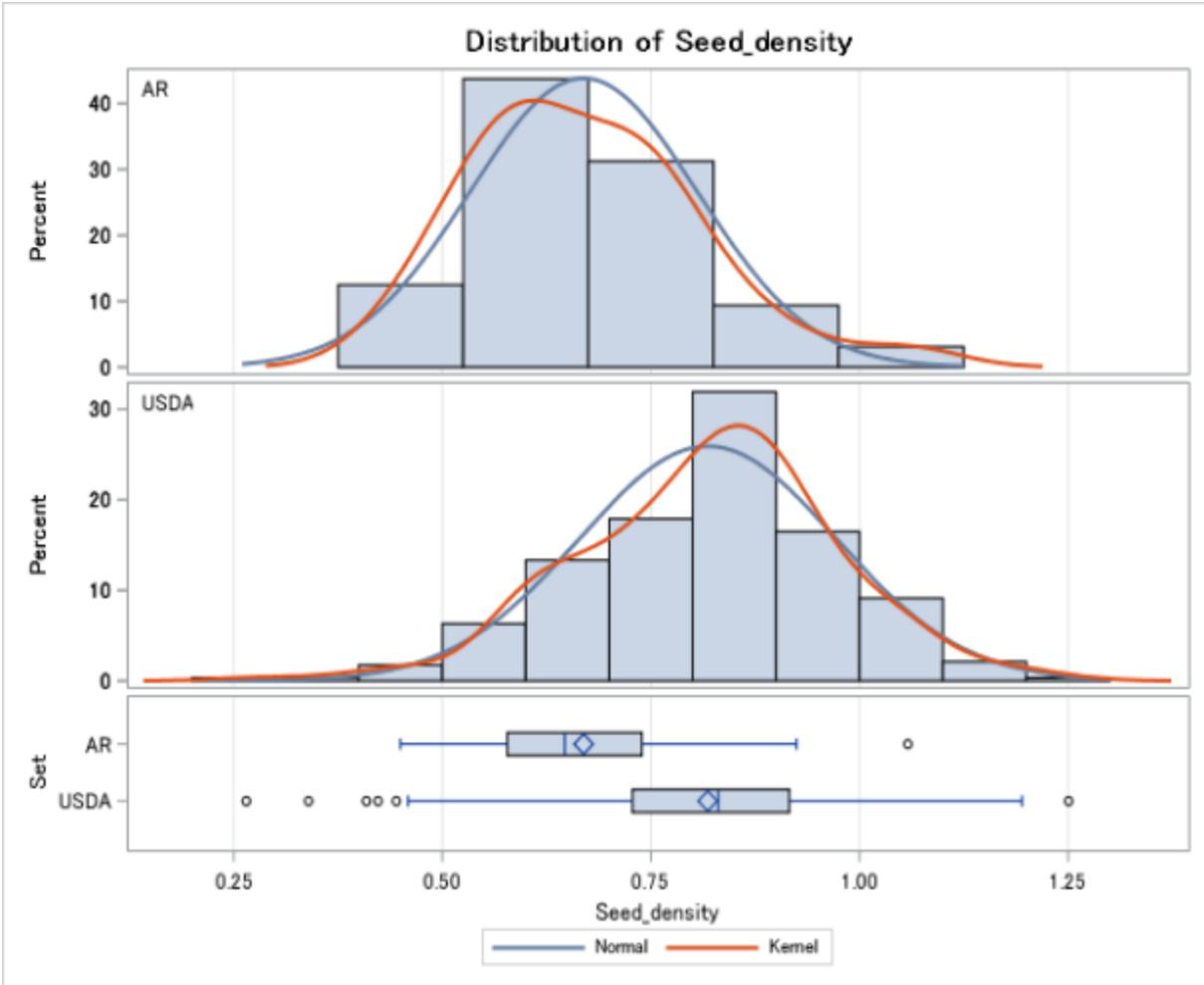


Figure 8. Distribution of AR breeding with USDA germplasm. The USDA germplasm have higher density or gram per cm of pod than AR breeding line. The difference in density is statistically significant.

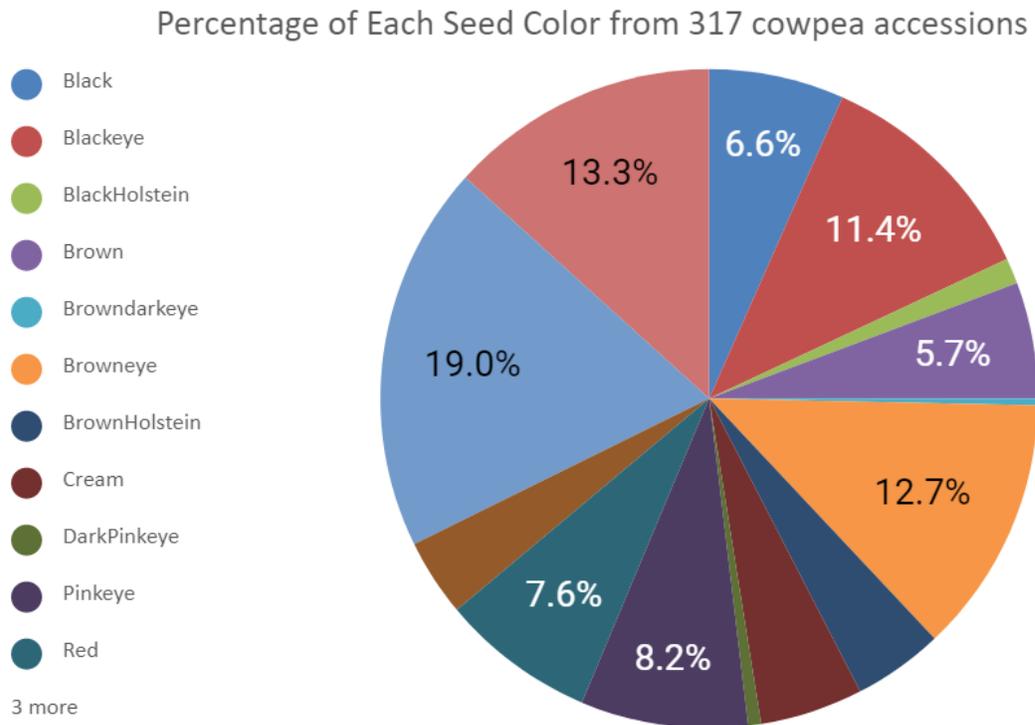


Figure 9. Pie chart showing the percentage of each color from 317 cowpea accessions

Percentage of USDA Seed Color from 285 lines

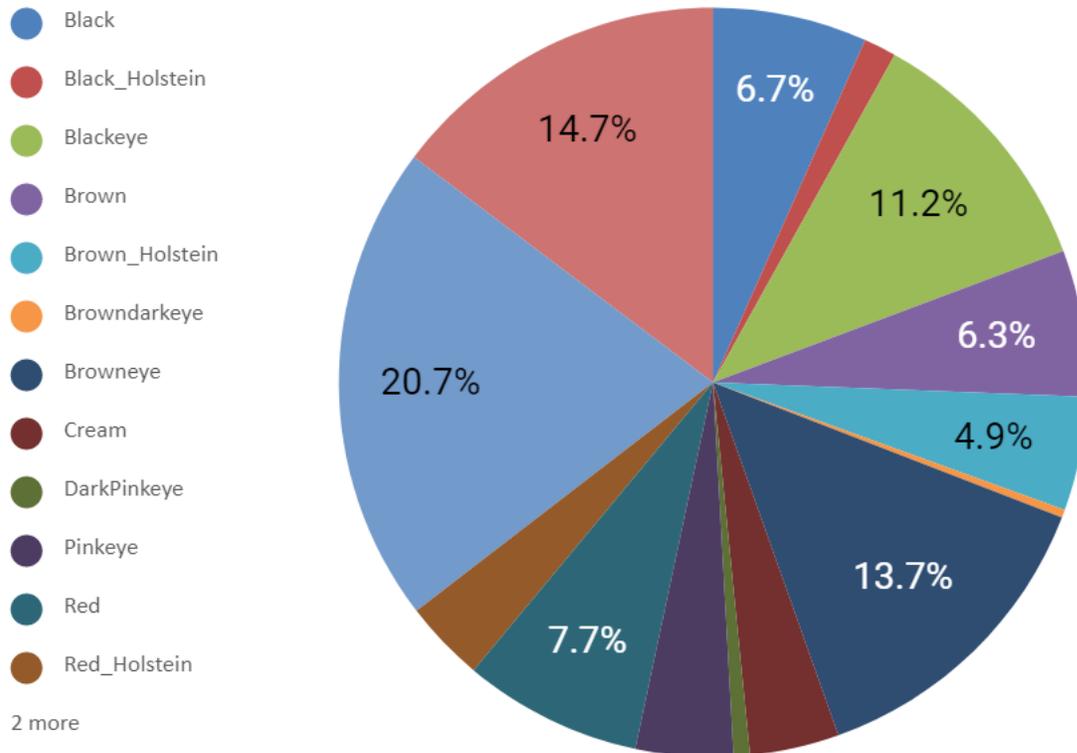


Figure 10. Pie chart showing the percentage of each color from 285 cowpea accessions.

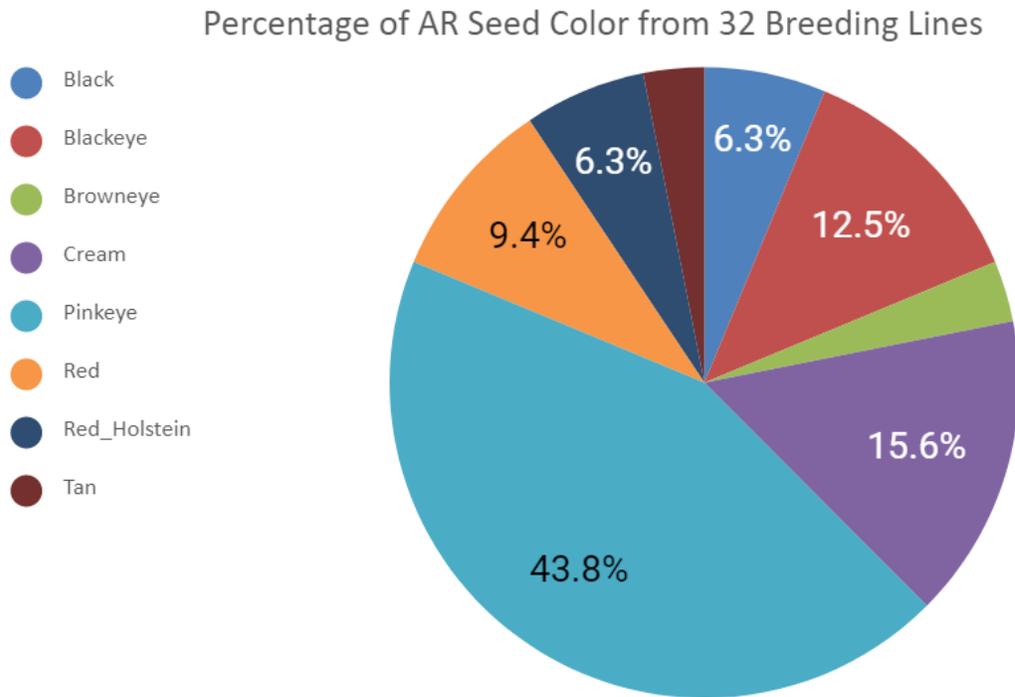


Figure 11. Pie chart showing the percentage of each color from 32 Arkansas breeding lines

Table 1

Cowpea breeding lines used in the experiment. 319 USDA germplasm accessions and 32 AR breeding lines

| Line ID |
|----------------|----------------|----------------|----------------|-----------------|
| 1_PI152195 | 71_PI354843 | 141_PI582735 | 211_PI592374 | 281_PI664518 |
| 2_PI152196 | 72_PI354855 | 142_PI582738 | 212_PI598335 | 282_IT89KD.288 |
| 3_PI152197 | 73_PI354860 | 143_PI582740 | 213_PI601085 | 283_IT84S.2049 |
| 4_PI154134 | 74_PI354864 | 144_PI582789 | 214_PI601453 | 284_CB27 |
| 5_PI165486 | 75_PI354865 | 145_PI582805 | 215_PI601682 | 285_IT82E.18 |
| 6_PI167284 | 76_PI354883 | 146_PI582809 | 216_PI610533 | 286_Suvita_2 |
| 7_PI180014 | 77_PI367921 | 147_PI582810 | 217_PI610604 | 287_IT00K.1263 |
| 8_PI186386 | 78_PI382123 | 148_PI582812 | 218_PI610620 | 288_IT84S.2246 |
| 9_PI190191 | 79_PI406285 | 149_PI582814 | 219_PI662992 | 289_IT93K-503-1 |
| 10_PI194213 | 80_PI406290 | 150_PI582815 | 220_PI662993 | 290_PI220851 |
| 11_PI200867 | 81_PI406292 | 151_PI582820 | 221_PI663011 | 291_PI293490 |
| 12_PI201498 | 82_PI406293 | 152_PI582824 | 222_PI663059 | 292_PI339588 |
| 13_PI202802 | 83_PI427093 | 153_PI582825 | 223_PI663101 | 293_PI339610 |
| 14_PI207527 | 84_PI430687 | 154_PI582850 | 224_PI663140 | 294_PI582415 |
| 15_PI218123 | 85_PI487518 | 155_PI582852 | 225_PI663148 | 295_PI582821 |
| 16_PI221730 | 86_PI491193 | 156_PI582855 | 226_PI663150 | 296_PI582984 |
| 17_PI221731 | 87_PI527263 | 157_PI582856 | 227_PI663151 | 297_PI583244 |
| 18_PI223023 | 88_PI527302 | 158_PI582857 | 228_PI664513 | 298_PI293491 |
| 19_PI227827 | 89_PI527561 | 159_PI582858 | 229_PI664514 | 299_PI354835 |
| 20_PI227829 | 90_PI527563 | 160_PI582861 | 230_PI664515 | 300_PI503326 |
| 21_PI227830 | 91_PI548785 | 161_PI582863 | 231_PI664517 | 301_PI582425 |
| 22_PI229734 | 92_PI578902 | 162_PI582864 | 232_PI664519 | 302_PI583232_2 |
| 23_PI229796 | 93_PI578911 | 163_PI582868 | 233_PI666253 | 303_PI608035 |
| 24_PI244571 | 94_PI579909 | 164_PI582874 | 234_PI666260 | 304_PI666251 |
| 25_PI250416 | 95_PI582340 | 165_PI582878 | 235_PI666262 | 305_PI75962 |
| 26_PI255765 | 96_PI582352 | 166_PI582894 | 236_PI666269 | 306_PI189374 |
| 27_PI255774 | 97_PI582353 | 167_PI582924 | 237_PI186360 | 307_PI194208 |
| 28_PI257463 | 98_PI582354 | 168_PI582926 | 238_PI194209 | 308_PI194210 |
| 29_PI279845 | 99_PI582417 | 169_PI582930 | 239_PI194211 | 309_PI227831 |
| 30_PI291140 | 100_PI582420 | 170_PI582931 | 240_PI220850 | 310_PI293486 |
| 31_PI292890 | 101_PI582421 | 171_PI582932 | 241_PI225922 | 311_PI354532 |
| 32_PI292892 | 102_PI582422 | 172_PI582942 | 242_PI253428 | 312_01.1781.1 |
| 33_PI292898 | 103_PI582423 | 173_PI582959 | 243_PI262179 | 313_07.303.2 |

34_PI292899	104_PI582465	174_PI582970	244_PI271256	314_9.105
35_PI292913	105_PI582466	175_PI583068	245_PI292894	315_9.109
36_PI293469	106_PI582467	176_PI583186	246_PI292897	316_9.175
37_PI293470	107_PI582468	177_PI583191	247_PI293487	317_09.295.10
38_PI293476	108_PI582469	178_PI583194	248_PI293582	318_9.393
39_PI293477	109_PI582474	179_PI583195	249_PI311119	319_9.47
40_PI293480	110_PI582512	180_PI583196	250_PI352997	320_9.481
41_PI293505	111_PI582521	181_PI583197	251_PI354531	321_9.529
42_PI293514	112_PI582522	182_PI583199	252_PI354673	322_9.655
43_PI293517	113_PI582523	183_PI583200	253_PI354760	323_9.671
44_PI293525	114_PI582525	184_PI583201	254_PI354762	324_9.686
45_PI293545	115_PI582530	185_PI583202	255_PI354854	325_09.692.5
46_PI293558	116_PI582541	186_PI583203	256_PI354878	326_9.697
47_PI293559	117_PI582542	187_PI583204	257_PI367861	327_9.702
48_PI293570	118_PI582554	188_PI583205	258_PI382111	328_09.714.14
49_PI293584	119_PI582566	189_PI583209	259_PI390421	329_9.741
50_PI293587	120_PI582567	190_PI583224	260_PI582428	330_09.749.5
51_PI293588	121_PI582569	191_PI583225	261_PI582551	331_15.07.86
52_PI300173	122_PI582570	192_PI583230	262_PI583190	332_AR95.348.4
53_PI304150	123_PI582571	193_PI583232_1	263_PI610520	333_ARBlackeye#1
54_PI312207	124_PI582572	194_PI583235	264_PI664524	334_ARBlackeye#1
55_PI312210	125_PI582573	195_PI583237	265_PI229551	335_EarlyAcre
56_PI339587	126_PI582575	196_PI583240	266_PI293500	336_EarlyScarlet
57_PI339591	127_PI582665	197_PI583241	267_PI382110	337_Ebony
58_PI339599	128_PI582667	198_PI583245	268_PI582402	338_Elegance
59_PI339600	129_PI582671	199_PI583247	269_PI582681	339_Empire.8
60_PI339603	130_PI582697	200_PI583248	270_PI582725	340_Empress.2
61_PI339605	131_PI582698	201_PI583249	271_PI582813	341_Encore.1
62_PI339609	132_PI582699	202_PI583250	272_PI582819	342_Envoy
63_PI339611	133_PI582702	203_PI583251	273_PI582822	343_EpicSelect.4
64_PI339613	134_PI582703	204_PI583274	274_PI582823	344_Epoch
65_PI349674	135_PI582727	205_PI583488	275_PI582853	345_ErectSet
66_PI353062	136_PI582729	206_PI583494	276_PI582865	346_ErectSetSelect.1
67_PI354499	137_PI582730	207_PI583513	277_PI582866	347_ExcelSelect.2
68_PI354580	138_PI582731	208_PI583550	278_PI582980	348_PI293568
69_PI354805	139_PI582733	209_PI583551	279_PI593117	349_PI353106

70_PI354832	140_PI582734	210_PI592369	280_PI642160	350_PI354666
				351_PI353270

Table 2

Descriptive statistics for 100- seed weight (g), seed density (g/cm of pod), pod length (cm), and seed number per pod for USDA germplasm accessions and Arkansas breeding lines

Set	Traits	N	Mean	Std Dev	Min	Max	CV†
All cowpea accessions§	100 seed weight (g)	317	13.63	3.89	6.03	29.00	28.54
	Seed density (g/cm of pod) ‡	317	0.80	0.16	0.26	1.25	19.74
	Pod length (cm)	317	16.74	2.83	11.14	30.50	16.89
	Seed number per pod	317	13.15	2.17	7.30	18.70	16.52
USDA genotypes	100 seed weight (g)	285	13.33	3.77	6.03	29.00	28.29
	Seed density (g/cm of pod)	285	0.82	0.15	0.26	1.25	18.81
	Pod length (cm)	285	16.66	2.85	11.14	30.50	17.08
	Seed number per pod	285	13.34	2.16	7.30	18.70	16.17
Arkansas genotypes	100 seed weight (g)	32	16.26	3.98	9.23	25.00	24.48
	Seed density (g/cm of pod)	32	0.67	0.14	0.45	1.06	20.39
	Pod length (cm)	32	17.47	2.58	11.16	21.95	14.77
	Seed number per pod	32	11.46	1.50	8.00	14.80	13.05

† CV refers to the coefficient of variation, which is the standard deviation divided by the mean.

‡ Seed density refers to the average of seed number per one-centimeter pod.

§All cowpea accessions consist of the USDA genotypes and the breeding lines from the University of Arkansas, Fayetteville.

Table 3

Comparison of 100 seed weight of USDA germplasm accessions and AR breeding lines

Method	Variations	DF	t Value	Pr > t
Pooled	Equal	315	4.14	<.0001
Satterthwaite	Unequal	37.519	3.97	0.0003

Table 4

Comparison of pod length of USDA germplasm accessions and AR breeding lines

Method	Variations	DF	t Value	Pr > t
Pooled	Equal	315	1.55	0.1215
Satterthwaite	Unequal	39.961	1.68	0.1009

Table 5

Comparison of seed number per pod of USDA germplasm accessions and AR breeding lines

Method	Variations	DF	t Value	Pr > t
Pooled	Equal	315	-4.85	<.0001
Satterthwaite	Unequal	45.406	-6.25	<.0001

Table 6

Comparison of seed density of USDA germplasm accessions and AR breeding lines

Method	Variations	DF	t Value	Pr > t
Pooled	Equal	315	-5.24	<.0001
Satterthwaite	Unequal	40.392	-5.77	<.0001

Table 7

Correlation analysis between 100-seed weight (g), seed density per 1-cm pod , pod length(cm), and seed number among 317 cowpea accessions, USDA genotypes, and Arkansas lines grown in the Fayetteville research station.

Set	Traits	100 seed weight (g)	Seed density (g/cm of pod)	Pod length(cm)	Seed number per pod
All Cowpea Accessions	100 seed weight (g)	1			
	Seed density (g/cm of pod)	-0.589 †	1		
	Pod length(cm)	0.513	-0.615	1	
	Seed number per pod	-0.231	0.631	0.148	1
USDA genotype	100 seed weight (g)	1			
	Seed density (g/cm of pod)	-0.548	1		
	Pod length(cm)	0.511	-0.607	1	
	Seed number per pod	-0.155	0.605	0.183	1
AR breeding lines	100 seed weight (g)	1			
	Seed density (g/cm of pod)	-0.706	1		
	Pod length(cm)	0.488	-0.742	1	
	Seed number per pod	-0.537	0.574	0.094	1

† Pearson's correlation coefficients calculated using JMP Genomics 9.

Table 8

Multicategory analysis using R-studio to infer the correlation between seed color and seed weight (g)

Set	Estimate †	Std. Error	z value	Pr(> z) ‡
Seed Weight:1§	-0.252	0.446	-0.564	0.573
Seed Weight:2	-0.115	0.442	-0.26	0.795
Seed Weight:3	0.270	0.441	0.612	0.54
Seed Weight:4	0.115	0.438	0.263	0.793
Seed Weight:5	-0.0231	0.455	-0.051	0.959
Seed Weight:6	-0.0974	0.442	-0.221	0.825
Seed Weight:7	-0.109	0.449	-0.241	0.809
Seed Weight:8	0.179	0.443	0.403	0.687
Seed Weight:9	-0.238	0.449	-0.53	0.596
Seed Weight:10	0.101	0.447	0.226	0.821
Seed Weight:11	0.930	0.470	0.198	0.843
Seed Weight:12	0.272	0.443	0.615	0.538
Seed Weight:13	-0.170	0.538	-0.316	0.752

† Estimates refer to the effect of the covariate seed weight on the logit response between a color category and a baseline category.

‡ Pr(>|z|) indicates the p-value to test for the estimate being zero.

§Indicates the regression response of the ith category and the baseline category.

Table 9

Multicategory analysis using R-studio to infer the correlation between seed color and seed density (g/cm of pod)

Set	Estimate †	Std. Error	z value	Pr(> z) ‡
Seed density:1§	-11.4	22.0	-0.52	0.603
Seed density:2	-6.54	21.7	-0.301	0.763
Seed density:3	-11.5	21.9	-0.525	0.599
Seed density:4	-6.01	21.6	-0.278	0.781
Seed density:5	-23.8	25.1	NA	NA
Seed density:6	-5.32	21.5	-0.247	0.805
Seed density:7	-6.95	22.4	-0.311	0.756
Seed density:8	-6.83	22.5	-0.304	0.761
Seed density:9	-8.62	22.2	-0.388	0.698
Seed density:10	-5.22	21.9	-0.238	0.812
Seed density:11	-8.18	24.1	-0.34	0.734
Seed density:12	-8.38	21.6	-0.389	0.697
Seed density:13	-47.3	41.8	NA	NA

† Estimates refer to the effect of the covariate seed density on the logit response between a color category and a baseline category.

‡ Pr(>|z|) indicates the p-value to test for the estimate being zero.

§Indicates the regression response of the ith category and the baseline category.

Table 10

Multicategory analysis using R-studio to infer the correlation between seed color and pod length (cm)

Set	Estimate †	Std. Error	z value	Pr(> z) ‡
Pod length:1§	0.0991	1.31	0.075	0.94
Pod length:2	0.0739	1.31	0.056	0.955
Pod length:3	-0.233	1.31	-0.177	0.859
Pod length:4	-0.1.83	1.31	-0.139	0.889
Pod length:5	-1.56	1.54	NA	NA
Pod length:6	0.0205	1.31	0.016	0.987
Pod length:7	-0.0716	1.35	-0.053	0.958
Pod length:8	-0.3.75	1.36	-0.277	0.782
Pod length:9	0.0875	1.33	0.066	0.947
Pod length:10	-0.154	1.33	-0.116	0.907
Pod length:11	-0.178	1.40	-0.127	0.899
Pod length:12	-0.0004.37	1.30	0	1
Pod length:13	-2.24	2.34	NA	NA

† Estimates refer to the effect of the covariate pod length on the logit response between a color category and a baseline category.

‡ Pr(>|z|) indicates the p-value to test for the estimate being zero.

§Indicates the regression response of the ith category and the baseline category.

Table 11

Multicategory analysis using R-studio to infer the correlation between seed color and seed number

Set	Estimate †	Std. Error	z value	Pr(> z) ‡
Seed number:1§	-0.177	1.35	-0.131	0.896
Seed number:2	-0.0444	1.34	-0.033	0.974
Seed number:3	-0.320	1.35	-0.237	0.813
Seed number:4	0.0940	1.34	0.07	0.944
Seed number:5	0.911	1.60	0.57	0.569
Seed number:6	-0.342	1.34	-0.256	0.798
Seed number:7	-0.0762	1.38	-0.055	0.956
Seed number:8	-0.0988	1.40	-0.071	0.944
Seed number:9	-0.0427	1.36	-0.031	0.975
Seed number:10	-0.276	1.36	-0.203	0.839
Seed number:11	-0.354	1.48	-0.239	0.811
Seed number:12	-0.571	1.34	-0.427	0.669
Seed number:13	2.01	2.65	0.757	0.449

† Estimates refer to the effect of the covariate seed number on the logit response between a color category and a baseline category.

‡ Pr(>|z|) indicates the p-value to test for the estimate being zero.

§Indicates the regression response of the ith category and the baseline category.