

Nutritional Qualities Evaluation of Ten Accessions of Cowpea (*Vigna unguiculata* L. Walp) Infected by Cercospora Leaf Spot Disease

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ABSTRACT

Background and Objective: Despite the importance of cowpea (*Vigna unguiculata* L. Walp) in Nigeria, its production in commercial quantity is constrained majorly by fungal diseases, especially Cercospora Leaf Spot (CLS). Therefore, a study was conducted to evaluate the nutritional qualities of ten cowpea accessions infected by CLS planted in the screen house **Materials and Methods:** The experiment was laid out in Completely Randomized Design with three replications. Data collected were on stomatal conductance, leaf chlorophyll content, disease evaluation and proximate analysis. The data collected were subjected to mixed model Analysis of Variance (ANOVA). Means of significant treatments were separated using Fisher Protected Least Significant Difference (LSD) at $p < 0.05$. **Results:** The effect of CLS on stomatal conductance, leaf chlorophyll content, disease evaluation and proximate analysis of the cowpea accessions differed significantly with TVU-9182 having the highest value of ash, crude fibre, moisture and fat at 3.92, 3.13, 15.67 and 4.48%, respectively with the value of carbohydrate at 51.99%. The TVU-9175 had the lowest value of ash, protein, moisture, crude fibre and fat at 2.17, 16.55, 8.67, 1.73 and 2.48%, respectively with the highest value of carbohydrate at 68.41%. The ten accessions were significantly susceptible ($p < 0.5$) to CLS with high susceptibility at 10 Weeks After Planting (WAP). **Conclusion:** The results showed that TVU-9174 and TVU-9182 had better nutritional qualities after CLS infection than the other infected cowpea accessions. Hence, TVU-9174 and TVU-9182 could be used as breeding materials based on the nutritional contents after CLS infection.

KEYWORDS

Susceptible, proximate, disease incidence, fibre, breeding

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INTRODUCTION

Cowpea is one of the important staple food in Africa and a major food crop in Nigeria. The cowpea crop has an average essential protein content of 25% and is rich in vitamins and minerals. A major food of the tropics with low fat and a major delicacy to humans and livestock, highly beneficial to human and animal health^{1,2}. It is estimated that 22% of the world's 141 Mha of cowpea is planted in rain-fed fields in West Africa, especially Nigeria^{3,4}, where Cercospora Leaf Spot Disease (CLSD) is a major cowpea disease that affects the balance in the synthesis of photosynthetic pigments resulting in poor quality and yield reduction³. These changes in the amounts of photosynthetic pigments in the cowpea plant due to the CLS

infection are closely associated with plant foliage lesion and death at the reproductive stage⁵⁻⁷. Hence, the ability to maintain the photosynthetic rate in the cowpea plant determines the disease tolerance⁸. Optimization of this physiological response to CLS to attain reproductive growth is a prerequisite for stable nutrients in cowpea seed development. This ability helps the plant to adapt to such limiting disease conditions^{8,9}. The physiological responses of the cowpea plant under disease conditions can affect the nutritional qualities of cowpea seeds produced. Hence, the objectives of this study were to investigate the effect of CLSD on the nutritional qualities of ten cowpea accessions.

MATERIALS AND METHODS

Study area: Screen house experiments were conducted in a Completely Randomized Design (CRD) with three replications at the Institute of Agriculture Research and Training (I.A.R and T) Obafemi Awolowo University, Ibadan, (longitude 3°15'N and latitude 7°23'E) at an altitude of 650 m above sea level. The annual rainfall of 1,320 mm with minimum and maximum annual temperatures of 20.1-23.6 and 27.1-34.5°C, respectively. The cowpea materials were obtained from the IITA gene bank and were selected based on various characteristics such as ease of tolerance, resistance, susceptibility to disease and yield. The cowpea materials used were ten different cowpea accessions in Table 1.

Research protocol: Pure *Cercospora* fungi culture isolated on Potato Dextrose Agar (PDA) from cowpea plants grown under natural field conditions was vortexed with 100 mL of sterilized distilled water. The concentration of the conidia suspension of the fungi was adjusted with a hemocytometer to 3×10^5 spore mL⁻¹ and later incubated for 3 days before it was inoculated onto the planted cowpea seedlings in the screen house damped with transparent cellophane cover for 24 hrs to provide a humid environment for the *Cercospora* fungus to thrive. A control experiment was also set up without any fungi inoculation to elucidate the standard nutritional content of the ten cowpea accessions.

The following physiological variables were taken after infection between the hrs of 9 am to 12 pm noon at 10 Weeks After Planting (WAP) with the control experiment.

Leaf stomatal conductance was measured using a leaf porometer¹⁰ (mmol m⁻² sec⁻¹) (leaf parameter model SC-1 LPS 1993) made in Japan.

Leaf chlorophyll was measured using SPAD meter¹¹ (Model 2900PDL) made in Italy.

Table 1: Sources of planting materials and their attributes

Cowpea accessions	Source	Characteristics
TVU-9100	IITA	The seed coat is smooth brown, the white eye with a round irregular-shaped pigmented area encircling the hilum
TVU-9106	IITA	The seed coat is a smooth white, white eye with a round irregular-shaped pigmented area encircling the hilum
TVU-9172	IITA	The seed coat is whitish-brown, the white eye with a round irregular-shaped pigmented area encircling the hilum
TVU-9174	IITA	The seed coat is white, the white eye with a round irregular-shaped pigmented area encircling the hilum
TVU-9175	IITA	The seed coat is speckled with the white and red, white eye with a round irregular-shaped pigmented area encircling the hilum
TVU-9179	IITA	The seed coat is smooth red, white eye with a round irregular-shaped pigmented area encircling the hilum
TVU-9182	IITA	The seed coat is white speckled with red, white eye with brown round irregular-shaped pigmented area encircling the hilum
TVU-9185	IITA	The seed coat is smooth red, white eye with a round irregular-shaped pigmented area encircling the hilum, small sized (3 cm)
TVU-9195	IITA	The seed coat is smooth brown speckled with red pigment, white eye with a round irregular-shaped pigmented area encircling the hilum
TVU-13664	IITA	The seed coat is white speckled with red pigment, white eye with a round irregular-shaped pigmented area encircling the hilum

Disease evaluation: Disease incidence and severity ratings were done visually at 10 WAP. The disease severity scoring scale was used on a scale of 0-5, where:

- 0 = No visible symptom
- 1 = Visible symptoms, <10% infection
- 2 = 11-20% infection
- 3 = 21-30% infection
- 4 = 41-50% infection,
- 5 = >50% infection¹²

To ascertain the level of infection in the plants and seeds. Disease incidence was calculated using plants showing symptoms of disease in each plot and expressed as a percentage of the total number of stands using the formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of healthy and infected plants}} \times 100$$

Seed yield and yield component variables: Seed weight (g) was weighed using a digital weighing balance (kg) (model: P-6202 made in Italy).

Harvesting and post-harvest operation: Harvesting was carried out when the pods of the cowpea plants turned to dry yellow from green colour. The pods were later threshed to obtain the seed grains and recorded as yield.

Proximate and nutrients quality determination: Moisture content, crude fibre, fat and ash content were determined using the method described by Njoku *et al.*¹³ while carbohydrate and crude protein content were determined¹⁴. Proximate analysis was determined on the infected harvested seeds and the control experiment with no *Cercospora* leaf spot disease to ascertain the cowpea nutritional quality.

Statistical analysis: Data collected were subjected To mixed model Analysis of Variance (ANOVA). Means of significant treatments were separated using Fisher Protected Least Significant Difference (LSD) at a 5% probability level.

RESULTS AND DISCUSSION

Effect of *Cercospora* Leaf Spot Disease (CLSD) at the reproductive growth stage (10 WAP) was evident in the stomatal conductance and leaf chlorophyll content in Table 2. A significant difference in cowpea accessions × disease severity was observed in the stomatal conductance, leaf chlorophyll content and the 100 g seed weight. The interaction effect between the cowpea accession and disease severity was not statistically significant. The mean total stomatal conductance (80.7 mmol m⁻² sec⁻¹) was observed in the CLSD infected cowpea accessions while the total mean for the control was 274.38 mmol m⁻² sec⁻¹. Thus, the highest stomatal conductance for the CLSD infected cowpea accessions was on TVU-9174 (115.9 mmol m⁻² sec⁻¹) accession while TVU-13664 (32.8 mmol m⁻² sec⁻¹) had the least significant stomata conductance. The leaf chlorophyll content of TVU-9174 (48.33) under optimum disease severity was significantly higher than those established under non-disease infection at the reproductive growth stage (Table 2). The cowpea accession: TVU-13664 had the least leaf chlorophyll content (37.00) when compared statistically under this disease condition as the highest leaf chlorophyll content (53.73) was also observed in TVU-9174 in the control experiment, which was significantly different from that of TVU-13664 (37.01) in the non-infected condition. A similar significant pattern of stomatal conductance and chlorophyll decline was observed across the cowpea accessions with disease incidence and severity.

Table 2: Effect of CLS disease infection at reproductive growth stage (10 WAP) on stomatal conductance and leaf chlorophyll content of the ten cowpea accessions

Cowpea accessions	Stomatal conductance (mmol m ⁻² sec ⁻¹)			Leaf chlorophyll content			Disease evaluation		
	Infected cowpea accessions		Mean	Infected cowpea accessions		Mean	Disease incidence 10 WAP	Disease severity 10 WAP	Weight of CLSD harvested 100 seeds (g)
	Control experiment			Control experiment					
TVU-9100	99.80 ^{de}	271.70 ^a	185.80	43.33 ^f	47.67 ^{ab}	45.50	30.00	2.02	18.51 ^b
TVU-9106	95.10 ^{ef}	262.00 ^b	178.10	45.00 ^{de}	47.67 ^{ab}	46.33	33.10	2.16	18.18 ^b
TVU-9172	80.40 ^f	278.60 ^a	179.50	45.33 ^{de}	46.00 ^{cd}	45.67	36.30	2.43	19.20 ^{ab}
TVU-9174	115.90 ^c	282.00 ^a	198.90	48.33 ^a	53.73 ^a	48.33	37.10	2.47	17.19 ^b
TVU-9175	83.20 ^f	279.00 ^a	181.10	45.00 ^{de}	46.00 ^{cd}	45.50	35.10	2.22	16.18 ^{bc}
TVU-9179	87.40 ^{ef}	271.40 ^{ab}	179.40	45.00 ^{de}	47.33 ^{abc}	49.17	38.50	2.89	17.15 ^b
TVU-9182	115.20 ^{cd}	284.90 ^a	200.05	47.33 ^{abc}	47.67 ^{ab}	47.50	36.10	2.39	19.13 ^{ab}
TVU-9185	81.10 ^f	283.10 ^a	182.10	44.00 ^{ef}	46.00 ^{cd}	45.00	34.70	2.18	20.01 ^a
TVU-9195	56.10 ^g	269.80 ^{ab}	162.95	44.00 ^{ef}	46.33 ^{bcd}	45.17	33.20	2.10	17.17 ^b
TVU-13664	73.90 ^{gh}	257.00 ^b	149.05	37.00 ^g	37.01 ^g	35.50	34.30	2.16	17.18 ^b
Means	80.70	274.38		40.82	45.94		32.40	1.00	
SED±	5.63			0.60			2.10		
Accessions (A)	**			**			**	**	
Disease severity (DS)	**			**			**	**	
A×DS	**			**			NS		

SED: Standard error of differences of means, alphabets that are the same across and along the table are not significantly different from each other at 5% probability level, **Significant at $p \leq 0.01$, NS: Not significant and 10 WAP: 10 weeks after planting

Table 3: Proximate analysis of the control experiment of the ten cowpea accessions

Parameters (%)	TVU-9100	TVU-9106	TVU-9172	TVU-9174	TVU-9175	TVU-9179	TVU-9182	TVU-9185	TVU-9195	TVU-13664
Ash	4.78	4.30	4.31	2.87	2.32	3.01	3.35	4.67	4.23	4.38
Protein	25.89	26.31	24.38	23.35	20.55	24.22	29.02	26.15	24.19	26.28
Moisture	17.23	14.28	14.07	11.59	8.88	11.01	13.32	15.21	16.45	13.45
Crude fibre	4.02	3.56	2.94	2.42	2.17	2.38	2.36	3.43	3.42	2.42
Fat	4.56	4.45	3.99	3.77	3.46	3.17	3.46	4.36	4.48	3.82
Carbohydrate	56.75	56.72	53.46	62.86	69.30	62.35	56.24	58.85	66.25	58.24
Phytate	0.78	0.79	2.03	1.45	1.22	1.92	0.79	0.92	1.03	0.59
Tannin	0.04	0.05	0.02	0.04	0.06	0.04	0.03	0.02	0.06	0.04
Oxalate	0.07	0.08	0.08	0.08	0.08	0.07	0.08	0.06	0.07	0.11
Saponin	0.30	0.11	0.04	0.04	0.32	0.02	0.22	0.56	0.38	ND

ND: Not detected

Proximate analysis result: The control experiment in Table 3 had high proximate values: The ash content had the highest value in TVU-9100 (4.78%) and the lowest in TVU-9175 (2.32%). The protein content was highest in TVU-9102 (29.02%) while the lowest protein value was recorded in TVU-9175 (20.55%). Moisture content was more in TVU-9100 (17.23%) while, TVU-9175 (8.88%) had the lowest moisture content. For the crude fibre, TVU-9100 (4.02%) had the highest value and TVU-9175 (2.17%) recorded the lowest value crude fibre. The TVU-9100 (4.56%) had a high-fat content while low-fat content was recorded in TVU-9179 (3.17%). The carbohydrate content recorded for TVU-9175 (69.30%) was highest while TVU-9172 (53.46%) had the lowest. However, for the anti-nutrient factors: The phytate content in TVU-9172 (2.03%) recorded the highest and lowest in TVU-13664 (0.59%). The tannin content was generally low in the ten cowpea accessions studied as TVU-9175 and TVU-9185 had both 0.06% tannin content, respectively and lowest percent values in TVU-9172 and TVU-9181 with 0.02%. The TVU-13664 (0.11%) had traceable oxalate with the lowest in TVU-9100, TVU-9179 and TVU-9195 with 0.07%, respectively. Saponin was not detected in TVU-13664 (ND) and the highest value of saponin content was recorded in TVU-9185 (0.56%).

The result of the proximate composition of the ten cowpea accessions harvested after CLSD infection is shown in Table 4.

Table 4: Proximate analysis of the ten cowpea accessions harvested after CLSD infection

Parameters (%)	TVU-9100	TVU-9106	TVU-9172	TVU-9174	TVU-9175	TVU-9179	TVU-9182	TVU-9185	TVU-9195	TVU-13664
Ash	3.92	3.33	3.42	2.75	2.17	2.67	2.75	3.58	3.67	2.67
Protein	20.81	21.04	19.29	19.14	16.55	19.54	25.02	21.15	20.09	24.22
Moisture	15.67	13.33	13.67	11.00	8.67	10.67	11.00	14.33	14.67	10.67
Crude Fibre	3.13	2.67	2.73	2.20	1.73	2.13	2.20	2.87	2.93	2.13
Fat	4.48	3.81	3.91	3.14	2.48	3.05	3.14	4.10	4.19	3.05
Carbohydrate	51.99	55.82	56.99	61.77	68.41	61.14	55.89	52.98	59.45	57.27
Phytate	ND	0.76	1.69	0.68	0.93	0.92	0.59	0.49	0.99	ND
Tannin	0.04	0.05	0.02	0.02	0.05	ND	0.00	ND	0.06	ND
Oxalate	0.07	0.08	0.08	0.08	0.08	0.07	0.08	0.06	0.07	0.11
Saponin	0.00	0.01	0.02	0.00	0.01	ND	0.01	0.02	0.00	ND

ND: Not detected

Ash content: The TVU-9100 (3.92%) had the highest ash content followed by TVU-9195 (3.67%) and TVU-9185 (3.56%), respectively while the least ash content value was TVU-9175 (2.17%). The high values of ash content recorded in these cowpea accessions indicated that the cowpea accessions are rich in minerals even after CLSD infection.

Crude protein content: The TVU-9182 (25.02%) had significantly higher protein content than TVU-13664 (24.22%), TVU-9185 (21.15%) than all the other cowpea accessions affected by CLSD with the least protein value obtained in TVU-9174 (11.00%). The variation in the cowpea protein content could be attributed to the genetic make-up response of the accessions used in this study and the CLSD infection.

Moisture content: The TVU-13664 (15.67%) cowpea accession had high moisture content than the moisture contents recorded in other cowpea accessions while the least moisture was recorded for TVU-9175 (8.12%). The high moisture content reduces the shelf-life of the cowpea accessions as low moisture content enhances a high storage quality, the high moisture content recorded in CLSD infected cowpea accessions from this study was attributed to it being freshly harvested.

Crude fibre content: The TVU-9195 (2.93%) cowpea accessions had a high percentage of crude fibre content than the other accessions affected by the CLSD, respectively. This was an indication that the cowpea accessions contained good digestible roughage fibre needed for a bowel movement and water absorption.

Fat content: The TVU-9100 (4.48%) had the highest fat content for the CLSD infected cowpea accessions while the lowest fat content was recorded for TVU-9175 (2.48%) though the fat content is of limited quality, hence, the fat content recorded in the CLSD affected cowpea accessions contributed to the palatability of the cowpea accessions used in this study.

Carbohydrate (CHO): The TVU-9175 (68.41%) was observed to have the highest percentage of CHO while the least carbohydrate content was obtained from TVU-9100 (51.99%). The carbohydrate content was observed to vary among the cowpea accessions. This implied that enough energy can be derived from the usage of the CLSD infected cowpea accessions used in this study.

Phytate: The TVU-9172 (1.69%) was of low phytate content while TVU-9185 (0.49%) had the lowest. However, phytate was not detected in TVU-9100 and TVU-13664. The low phytate recorded in the accessions implied that they were used in defence response against *Cercospora* leaf spot infection. The presence of phytate and anti-nutritional content in the cowpea accessions acted as an immune enhancer in response to CLSD on the foliage¹⁴.

Tannin: The tannin content recorded was significantly low for all the ten cowpea accessions. The TVU-9195 (0.06%) had the highest tannin content closely followed by TVU-9175 and TVU-9106 with 0.05%, while the least tannin content was detected in TVU-9182 (0.00) it was however not detected in TVU-9179, TVU-9185 and TVU-13664. The presence of tannin in the cowpea act as a suppressor against CLS infection.

Oxalate: The oxalate content of the ten cowpea accession infected by CLSD ranged between 0.11-0.06%. where TVU-13664 (0.11%) had the highest percentage and the least percent was recorded in Tvu-9185 (0.06%). Oxalate occurs naturally in cowpea legume plants and assists in defence response but they have little or no useful effect on human health through high level causes flatulence and constipation in man.

Saponin: The low saponin content of the ten cowpea accessions affected by CLSD varied from 0.02-0.00%. The TVU-9172 and TVU-9185 cowpea accessions had the highest level of saponin (0.02%) while it was rarely found in TVU-13664 (ND). The low presence of saponin accounted for the susceptibility of the cowpea to *Cercospora* leaf spot infection. This is because Saponin possesses anti-microbial activities that control fungal infections^{14,15}.

The result in Table 3 and 4 (the infected cowpea and the control) when compared. Table 4 shows the effect of *Cercospora* leaf spot disease on nutrition and the anti-nutritional composition of the ten cowpea accessions. The TVU-9100 showed the highest value of Ash at 3.92 while TVU-9175 had the lowest value of Ash at 2.17. Protein was highest in TVU-9182 at 25.02 and lowest at TVU-9175 at 16.55. The TVU-9100 recorded the highest value of moisture content at 15.67 while TVU-9175 had the least moisture content of 8.67. Crude fibre (CF) was highest in TVU-9100 with a value of 3.13 and lowest at TVU-9175 with a value of 1.73. Fatwas werealso highest at TVU-9100 with a value of 4.48 and lowest at TVU-9175 with a value of 2.48. The TVU-9175 showed the highest value of carbohydrate at 68.41 while TVU-9100 had the lowest value of carbohydrate at 51.99. Phytate was highest in TVU-9172 at 1.69 and lowest at TVU-9185 at 0.49. The TVU-9195 recorded the highest value of Tannin at 0.06 while TVU-9182 had the least Tannin of 0.00. Oxalate was highest in TVU-13664 with a value of 0.11 and lowest at TVU-9185 with a value of 0.06. Saponin was also highest at TVU-9172 and TVU-9185 with a value of 0.02 and lowest at TVU-9100, TVU-9174 and TVU-9195 with a value of 0.00 when compared to the result in the control experiment. (Table 3). The result was in-line with what was observed based on the susceptibility of the ten different accessions of cowpea to the *Cercospora* leaf spot disease.

DISCUSSION

The lack of empirical data for the response of cowpea accessions to infection by *Cercospora* Leaf Spot Disease (CLSD) and its effects on nutritional qualityis one of the factors limiting the progress of breeding of cowpea for nutritional improvement in Nigeria and the Sub-Sahara African States. From this study, the results showed that TVU-9185 and TVU-9172 had significantly the highest leaf photosynthetic rate, stomatal conductance which was evident in the plant physiological response to CLSD on the harvested seeds after CLSD infection. This gave a significant difference ($p < 0.05$) in the cowpea accessionsused while the least value was observed in TVU-9175. The stomatal conductance generally decreased with the disease severity and incidence, the tendency of reduction of stomatal conductance under the disease incidence and severity is consistent with observations made on photoinhibition on plant^{16,17}. A reduction in the photosynthetic rate was more pronounced in the CLSD infected cowpea plant than in the control. This result agrees with the observations^{17,18} that the reduction in the leaf photosynthetic absorption was a result of the infection of CLSD through its circumvention of free oxygen needed by the cowpea plant to carry out photosynthesis this was evidenced by the clear downward record of the stomata conductance that created a metabolic bottleneck on the plant ability to use chlorophyll and sunlight^{6,19}. Thus, significant cowpea accessional difference in the net photosynthesis was observed in the infected cowpea accessions. Thus, TVU-9174 and TVU-9182 showed higher photosynthetic rates compared to TVU-13664. This implied

that TVU-9174 and TVU-9182 accessions are fairly more tolerant to the CLSD infection than TvU-13664 and can photosynthesize under certain levels of disease response as similar observations were emphasized²⁰⁻²⁵. The findings from this study showed that the exposure to CLSD affected the photosynthetic rate and in turn affected the nutrient uptake heavily resulting in a decline in growth rate and seed formation at the reproductive stage of the affected cowpea accessions evidenced in the nutritional content²⁰⁻²⁵. The change in the nutritional quality was a result of the infection caused by CLS disease by the inhibition of the photosynthetic activity of the cowpea plant as the plants managed to attain reproductive growth for seed production, thus, affecting the nutrient quality and yields.

From this study, the result of disease incidence and severity showed that the *Cercospora* leaf spot disease affected the proximate composition differently which could be a result of the differences in their level of inherent genetic resistance and tolerance which corroborated the findings²⁶ which also had the same results on disease incidence and severity of *Cercospora* leaf spot on the groundnut plant. The proximate analysis result of the CLSD infected cowpea accessions and the control showed that CLS has a significant effect on the nutritional qualities and anti-nutrition composition of the ten cowpea accessions tested on proximate analysis such as protein, ash, moisture, crude fibre fat carbohydrate, phytate tannin, oxalate and saponin as compared in Table 3 and 4.

CONCLUSION

From this study, *Cercospora* Leaf Spot (CLS) inhibited the physiological functions of the reproductive growth of the cowpea plants as evidenced in the low stomatal conductance, decreased photosynthesis and changes in the nutritional qualities of the ten cowpea accessions infected by *Cercospora* Leaf Spot Disease (CLSD).

This study also revealed that TVU-9174, TVU-9182 and TVU-9185 cowpea accessions had a better adaptive response to physiological changes and the reduction in their nutritional qualities was induced by CLSD. This is an advantage to farmers and breeders as these accessions could be recommended as breeding materials for their physiological tolerance to CLS infection and nutritional qualities. Hence, more research is still recommended to evaluate the response of cowpea plants to CLSD dynamics and its influence on the nutritional quality of cowpea accessions.

SIGNIFICANCE STATEMENT

This study discovers the influence of *Cercospora* leaf spot disease on the nutritional quality of cowpea (*Vigna unguiculata*) that can be beneficial for both cowpea breeders, farmers and consumers. Hence, this study will help the researcher to compare the nutritional qualities of susceptible cowpea infected by CLSD and recommend them as breeding materials for farmers to ensure food security and improved cowpea germplasm which is critical to cowpea production that many breeders have ignored and have not been explored. Thus, the best cowpea biotechnology practice is to improve the nutritional quality and palatability of cowpea accessions affected by CLSD to boost their nutritional content, subsequent yield increase and resistant accessions to the disease may be arrived at.

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