



**Full Length Article**

## Genetic Analysis Indicates a Relationship of Cowpea (*Vigna unguiculata* (L.) Walp.) Accessions from Oman to Cowpea in the Indian Subcontinent

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### Abstract

Cowpea is a widely grown crop in different parts of the world, including Oman which is located in South East Arabia. Since this part of the world has long been a trade centre connecting the Arabian Peninsula with the Indian Subcontinent and South East Africa, little is known about the diversity of cowpea accessions in this area. A study was conducted to characterize and evaluate 25 cowpea accessions collected from six geographical areas (six populations) in Oman using morphological and amplified fragment length polymorphism (AFLP) analyses. The mean standardized Shannon-Weaver diversity index ( $H'$ ) was found to be 0.65 for quantitative and 0.55 for qualitative traits, indicating the presence of variation in morphological traits among accessions. AFLP analysis indicated the presence of moderate levels of genetic diversity (0.214-0.316) within the 25 cowpea accessions, while the level of genetic diversity was relatively higher (0.355-0.385) within populations from the six regions, indicating the presence of variation among accessions from the same region. Analysis of molecular variance (AMOVA) indicated the presence of a very low level of genetic differentiation ( $F_{ST} = 0.032$ ) among Omani accessions. Morphological and molecular data imply that cowpea has been grown in Oman for a relatively long period of time. Variation in the cowpea germplasm in Oman indicates that some accessions may carry important traits worth of investigation in future breeding programs. © 2018 Friends Science Publishers

**Keywords:** AFLP; Evolution; Plant breeding; Population structure

### Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important grain legume in different parts of the world (Singh *et al.*, 1997; Dube and Fanadzo, 2013; Boukar *et al.*, 2016). Its grains are rich in protein contents and gained the title of “poor man’s meat” in developing world (Pengelly and Maass, 2001; Gonçalves *et al.*, 2016). Moreover, the crop can also perform ecosystem services by fixing nitrogen and thus decreases the nitrogen requirement in double crop rotation systems (Sanginga, 2003). Its annual production is almost 7 million metric tons a year (FAO, 2017).

Selection for high yielding genotypes through various breeding procedures and farmer choice for few elite cultivars over the indigenous land races have reduced the genetic diversity in the field crops (Rauf *et al.*, 2015). Landraces tend to have high levels of diversity and thus have higher phenotypic plasticity to tolerate biotic and abiotic stresses compared to commercial varieties.

However, most of these landraces are at the verge of extinction due to their lower performance under high input conditions and alternative farmer priorities. High levels of genetic diversity in populations act as buffer against the environmental and biotic stresses (Al-Sadi *et al.*, 2012; Al-Mamari *et al.*, 2014).

Molecular markers have been widely exploited to characterize germplasm and have been considered more useful than morphological and protein-based markers due to their ability to detect high level of polymorphism in closely related accessions (Rauf *et al.*, 2010; Andargie *et al.*, 2014; Pottorff *et al.*, 2014; Gerrano *et al.*, 2015; Boukar *et al.*, 2016; Muranaka *et al.*, 2016). The AFLP technique is considered one of the most appropriate molecular tools for fingerprinting and has been exploited to characterize cowpea and other crops (Coulibaly *et al.*, 2002; Ghalmi *et al.*, 2010). Analysis of diversity using phenotypic traits is also widely practiced for cowpea and other crops (Ghalmi *et al.*, 2010; Muranaka *et al.*, 2016).

Oman is located in the South East of the Arabian Peninsula, facing the Indian Ocean. It had been known since prehistoric times as a center of trade connecting the Arabian Peninsula with the Indian subcontinent and south east Africa. This has led to the introduction of several crop species from these areas into Oman, making Oman a center of diversity of some important plant species (Davies and Albrigo, 1994; Pickering and Patzelt, 2008; Brinkmann *et al.*, 2009; Al-Abbasi *et al.*, 2010; Brinkmann *et al.*, 2011; Patzelt, 2011; Patzelt *et al.*, 2014).

Cowpea has been cultivated by local farmers in Oman for hundreds of years. Due to the lack of information about this crop in this part of the world, a study was carried out to characterize 25 Omani cowpea accessions using phenotypic characteristics and AFLP fingerprinting. Characterization of genotypes will help to select parental lines to be used to develop appropriate breeding strategies to improve cowpea production.

## Materials and Methods

### Collection of Samples

Cowpea accessions were collected from diverse localities in six geographical regions of Oman: Al-Sharqiya, Al-Batinah, Al-Dakhiliya, Al-Buraimi, Dhofar and Al-Dhahira, with the distance between locations ranging from 10 to 1000 km (avg. 200 km) (Fig. 1). A core sub set of 25 accessions were chosen for morphological and molecular characterization of genetic diversity in the germplasm. The core subset was augmented by five populations of cowpeas from India (2), Pakistan (1), Myanmar (1) and Australia (1) for comparison using molecular characterization.

### Growth Conditions

Cowpeas germplasm was raised inside a temperature-controlled glasshouse at Agriculture Experiment Station (AES), Sultan Qaboos University (SQU) under the following conditions: 26°C temperature, natural sun light and 60-70% relative humidity. Seeds of each accession were sown in seedling trays containing sterilized potting soil. Two-week old seedlings were transplanted to 25 × 20 cm plastic pots (1 seedling/pot) filled with sterilized peat moss and perlite in the ratio of 2:1 for morphological evaluation.

The experiment was carried out in a completely randomized design. The plants were irrigated for three min using drip irrigation every alternate day in the early morning from planting until harvesting. NPK Fertilizer (20:20:20) was applied at a rate of 20 kg ha<sup>-1</sup> throughout the growth period as plant's requirement.

### Measurement and Statistical Analysis of Morphological Traits

Accessions were evaluated for 23 traits according to the standard descriptors for cowpea established by the International Board for Plant Genetic Resources (Table 1). The quasi-quantitative traits such as number of main

branches, number of racemes per plant, number of pods per plant, number of pods per peduncle, seed width (cm) and seed length (cm) were either manually counted or measured with scale.

Quantitative and qualitative morphological characters were analyzed according to the Ward's method (Ward Jr, 1963) using the "R" program (Hammer *et al.*, 2001). Cluster tree was produced using the Ward's method based on the genetic distance among accessions. Shannon's diversity index (H') was calculated manually among characteristics (Margalef, 1958).

### Amplified Fragment Length Polymorphism (AFLP) Analysis

About 0.1 g of leaf tissue was collected for total genomic DNA extraction according to the Cetyl trimethylammonium bromide (CTAB) procedure described by Doyle and Doyle (1990) with slight modifications. AFLP analysis was carried out according to the method described by Vose *et al.* (1995). The analysis included bulked DNA from 6 regions, 75 samples representing 25 Omani cowpea accessions (3 replicates for each accession), and 5 commercial varieties from India, Pakistan, Myanmar and Australia. Six primer sets were used in the analysis: E+AAC/M+CAG, E+AAC/M+CTA, E+ACT/M+CAG, E+ACG/M+CAC, E+ACG/M+CTA, and E+AGG/M+CAC. AFLP raw data generated by the ABI Sequencer Prism was analyzed by the GeneMapper 4.0 software.

### Genetic Distance and Cluster Analysis

AFLP bands (between 50 to 500 bp) were scored as present (1) or absent (0). Analysis of Molecular Variance (AMOVA) was used to examine genetic differentiation among cowpea accessions and to calculate gene flow. The Cluster tree analysis was done based on similarity matrices using Jaccard Coefficient of Genetic Similarity (JCGS) (Sneath and Sokal, 1973). A dendrogram was constructed by using UPGMA method. Nei's gene diversity (Nei, 1973) and genetic distance (Nei, 1978) were determined using "R" language function.

## Results

### Phenotypic Diversity within Cowpea Germplasm

Twenty-five accessions of cowpeas collected from six agro-ecological regions of Oman were scored for 23 morphological traits to estimate phenotypic diversity within and between accessions. Standardized diversity index (H') for quantitative characters ranged from 0.31 to 0.88 (Table 2). The highest diversity index for quantitative characters was found in the number of racemes plant<sup>-1</sup> and seed crowding (Table 3). H' varied between 0.0 (mono-morphic) to 0.99 (polymorphic) for qualitative traits (Table 4). Overall, the average H' of all qualitative traits was 0.55 (Table 3).

**Table 1:** Morphological characters used to characterize cowpea accessions and methods of scoring/ measurement

Serial No.	Morphological Characters	Method of Measurement	Scoring/Measurement scale	Nature of character
1	Growth habit	Visual assessment	Acute-erect=1, Erect=2, Semi-erect=3, Intermediate=4, Semi-postrate=5	Qualitative
2	Leaf marking	Visual assessment	Absent=0, Present=1	Qualitative
3	Twinning tendency	Visual assessment	None=0, Slight=3, Intermediate=5, Pronounced=7	Qualitative
4	Pod attachment to peduncle	Visual assessment	Pendant=3, 30-90 Down from erect=5, Erect=7	Qualitative
5	Immature pod pigmentation	Visual assessment	Pigmented tip=1, Pigmented sutures=2	Qualitative
6	Pod pigmentation	Visual assessment	Pale tan=1, Dark tan=2, Dark brown=3	Qualitative
7	Raceme position	Visual assessment	Mostly above canopy=1, In upper canopy=2	Qualitative
8	Pod curvature	Visual assessment	Straight=0, Slightly curved=3	Qualitative
9	Flower Color	Visual assessment	White=1, Violet=2	Qualitative
10	Seed shape	Visual assessment	Kidney=1, Rhomboid=5	Qualitative
11	seed eye color	Visual assessment	Eye absent=0, Tan brown=2	Qualitative
12	Plant pigmentation	Visual assessment	Moderate at the base and tips of petioles=3, Intermediate=5, Extensive=7	Qualitative
13	Leaf color	Visual assessment	Intermediate green=5, Dark green=7	Qualitative
14	Terminal leaflet shape	Visual assessment	Globose=1, Sub-globose=2, Sub-hastate=3, Hastate=4	Qualitative
15	Plant hairiness	Visual assessment	Glabrescent=3, Short appressed hairs=5, Pubescent to hirsute=7	Qualitative
16	Leaf texture	Visual assessment	Coriaceous=1, Intermediate=2, Membranous=3	Qualitative
17	Number of main branches	Counted	-	Quantitative
18	Number of racemes/ plant	Counted	-	Quantitative
19	Number of pod / plant	Counted	-	Quantitative
20	Number of pod / peduncle	Counted	-	Quantitative
21	Seed width	Measured (cm)	-	Quantitative
22	Seed length	Measured (cm)	-	Quantitative
23	Seed crowding	Visual estimation	Semi-crowded=3, Crowded=5	Qualitative

Polymorphism was evident in morphological traits for color, shape and size of seeds and leaves. There were two types of leaf shapes: globose which was found only in Dhofar accessions and hastate which was found in accessions from the North. Seed shapes were found to be of two types: rhomboid shape and kidney shape. On the other hand, three different types of seed color: black and red were found in accessions from Dhofar region and tan brown color was found in accessions from the North of Oman.

#### Cluster Analysis According to Morphological Data

Analysis of cowpea accessions from six geographical regions in Oman showed that all accessions from Dhofar (South of Oman) clustered in one group. The terminal leaf shape of accessions from Dhofar was sub-globose, which is different from most of the other accessions. In addition, accessions from Dhofar have coriaceous and membranous leaf shape, which is different from all the other accessions. Accessions from Sohar and Saham grouped according to their geographic origin, and they had dark brown pods. However, accessions from other regions clustered together, with no relationship with the regions from which they came from (Fig. 2).

#### AFLP Analysis

A total of 3057 alleles were detected with 6 *EcoRI/MseI* primer combinations. The number of alleles per primer pair ranged from 491 to 545 (avg. 514). The percentage of polymorphic loci for the six primer combinations ranged from 97.4 to 99.8% (avg. 99.0%) (data not shown). The highest estimates of Nei gene diversity was found in primer-pair combinations E+AAC/M+CAG (0.285) and E+AAC/M+CTA (0.282).

**Table 2:** Shannon weaver diversity index (H') (Shannon and Weaver, 1949) and phenotypic frequencies (classes) of quantitative traits observed for Omani cowpea accessions

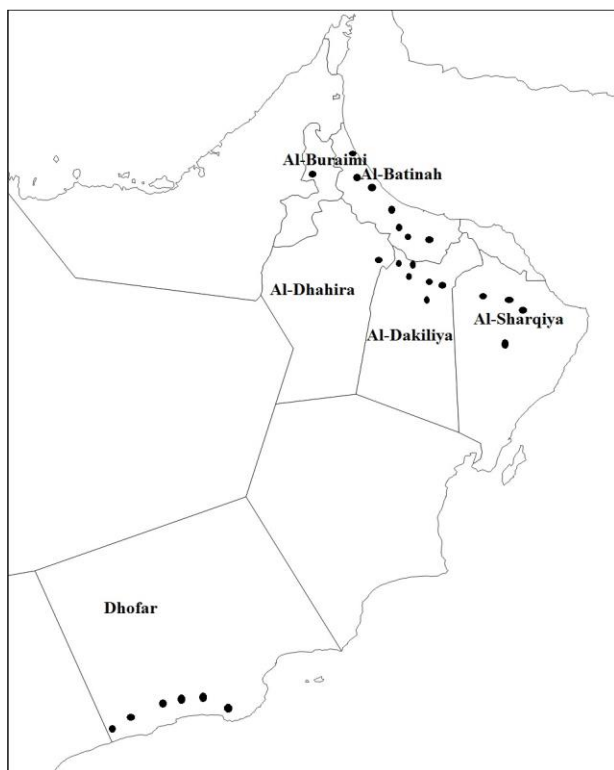
Quantitative traits	Number of Classes	(H')
No. of racemes plant <sup>-1</sup>	4	0.88
Seed crowding	2	0.80
No. of main branches	3	0.77
Seed width	3	0.65
Seed length	4	0.57
No. of pod plant <sup>-1</sup>	5	0.57
No. of pod peduncle <sup>-1</sup>	3	0.31
Average		0.65

**Table 3:** Shannon weaver diversity index (H') (Shannon and Weaver, 1949) and phenotypic frequencies (classes) of qualitative traits observed for Omani cowpea accessions

Qualitative Traits	Number of Classes	(H')
Seed shape	2	0.99
Leaf texture	3	0.97
Plant hairiness	3	0.94
Terminal leaflet shape	4	0.94
Pod pigmentation	3	0.89
Twinning tendency	4	0.79
Leaf color	2	0.70
Pod curvature	2	0.65
Pod attachment to peduncle	2	0.54
Growth habit	5	0.52
Plant pigmentation	3	0.35
Raceme position	2	0.31
Immature pod pigmentation	2	0.21
Leaf marking	2	0.0
Flower color	2	0.0
Seed eye color	2	0.0
Average		0.55

#### Genotypic and Genetic Diversity

AFLP analysis using 2335 polymorphic loci of 25 Omani cowpea accessions produced 25 AFLP genotypes.



**Fig. 1:** Map of Oman indicating the distribution of cowpea accessions collected from six different geographical regions in Oman

The percentage of polymorphic loci ranged from 66.7% to 99.5% (Table 4). Gene diversity estimates were in the range of 0.355 for Al-Buraimi to 0.385 for Al-Dhahira (Table 4).

#### Cluster Analysis Based on AFLP Analysis

Cluster analysis of cowpea populations separated cowpea populations into two main groups (Fig. 3). The first group included populations from Dhofar, Al-Dakhiliya, and Al-Dhahira, while the other one included two sub-groups, with populations from Al-Buraimi, Al-Batinah and Al-Sharqiya clustering more closely to populations from Australia, India, Myanmar and Pakistan. One accession from India clustered separately from the others (Fig. 3). No morphological traits were related to the molecular grouping of the accessions. Al-Dakhiliya and Al-Dhahira, which clustered together, are geographically close to each other.

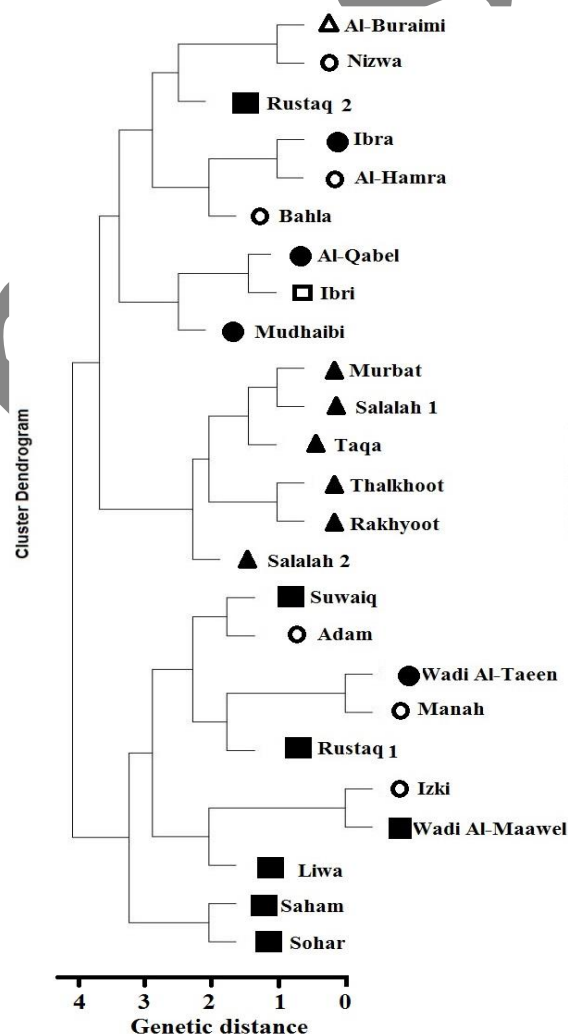
#### Partition of Genetic Variation Among and within Omani Cowpea Populations

Analysis of molecular and morphological variance (AMOVA) showed that most genetic variation exists within populations of cowpea, with only 3.2% of genetic variation being among the different populations.

**Table 4:** Population genetic analysis of Omani cowpea from different geographical origins based on AFLP fingerprinting using 2335 polymorphic loci

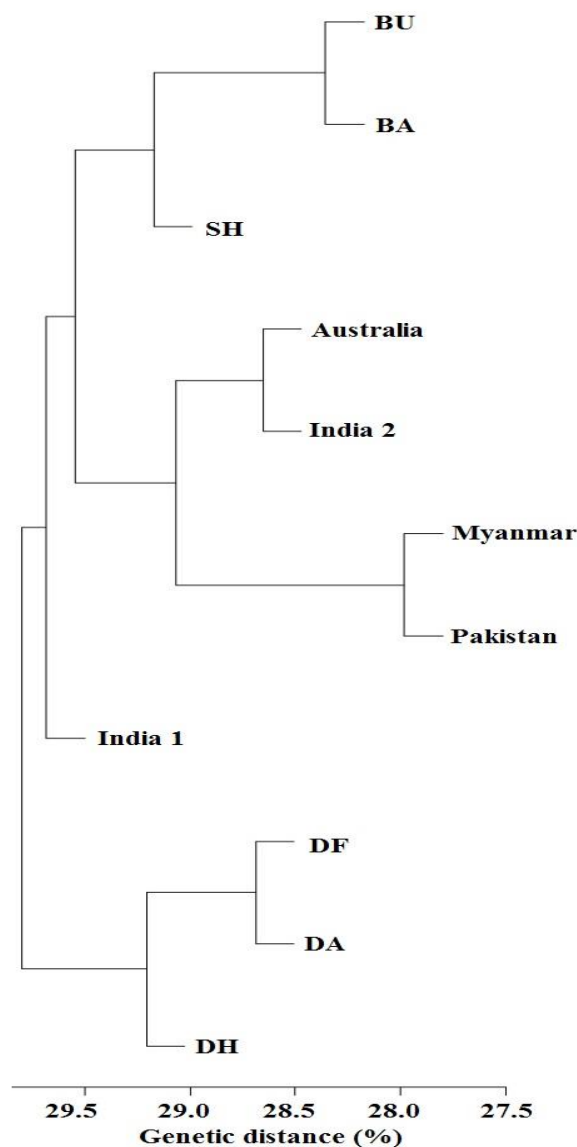
Population (region)	N	NPL	PPL	g	H
Al-Batinah (BA)	21	978	99.5	21	0.360
Al-Dakhiliya (DA)	18	978	99.5	18	0.359
Al-Dhahira (DH)	3	656	66.7	3	0.385
Al-Sharqiya (SH)	12	971	98.8	12	0.371
Al-Buraimi (BU)	3	672	68.4	3	0.355
Dhofar (DF)	18	968	98.5	18	0.368
Total	75	5223	-	75	0.366

N= samples sizes; NPL= Total polymorphic loci; PPL= percentage of Polymorphic loci; g=number of different genotypes recovered and H= Nei (1973) gene diversity



**Fig. 2:** UPGMA dendrogram illustrating Nei genetic distance among 25 Omani cowpea populations obtained from 6 districts based on morphological traits

However, higher morphological differentiation was noted among the accession (37%). The mean level of gene flow among the different populations, estimated from AFLP alleles, was high (7.56).



**Fig. 3:** UPGMA dendrogram illustrating regional genetic diversity among cowpea populations obtained from 6 regions *i.e.*, Al-Dhahira (DH), Al-Dakhiliya (DA) and Dhofar (DF), Al-Sharqiya (SH), Al-Batinah (BA) and Al-Buraimi (BU) compared with exotic germplasm based on AFLP molecular marker analysis using 2335 polymorphic alleles

## Discussion

Cowpea is considered one of the traditional crops that had been cultivated in many regions of Oman in the past. Our finding showed that there is high variation in quantitative traits such as the number of racemes per plant. Overall, results showed that variation in quantitative traits was higher than qualitative traits. This may be due to the low level of polymorphism in many of

qualitative traits (Manggoel and Uguru, 2011). However, polymorphism revealed through qualitative traits is more reliable since quantitative traits are subjected to environmental variation.

AFLP markers analyses produced 3057 polymorphic loci (99%) through six primer combinations. Analysis of genetic diversity within populations of Omani cowpea showed that Omani populations have moderate levels of genetic diversity (mean = 0.292). The level of genetic diversity of the Omani accessions is higher compared cowpea in other countries (Vir and Singh, 2014; Gerrano *et al.*, 2015). For instance, gene diversity levels of 0.164 and 0.173 were reported in Italian accessions and African accessions of cowpea, respectively (Coulibaly *et al.*, 2002). Ba *et al.* (2004) reported gene diversity levels of 0.223 for 30 cowpea accessions from West, East and southern Africa. Thus Omani accessions adapted to local conditions still maintain higher level of genetic diversity than some other regions of the world. Farmers in Oman commonly select their own seeds and conserve landraces and local cultivars for planting next year. However, informal exchange strategy of seeds between farmers is common in Oman.

Analysis of genetic diversity showed that Al-Dhahira and Al-Sharqiya have the highest level of gene diversity. This may provide evidence of two major centers of diversity for cowpea in Oman. Populations from these areas may be conserved as core collections. The six accessions from Dhofar formed a tight cluster away from all other accessions. This may indicate, as it is shown in this study, that these accessions possess some morphological characteristic that distinguish them from all other accessions. Dhofar is in the south of Oman and it is separated from other regions by more than 700 km by a desert (the Empty Quarter). The exchange of germplasm is expected to be limited compared to the exchange of germplasm among regions in the northern part of the country.

Omani accessions from Al-Buraimi, Al-Batinah and Al-Sharqiya were placed close to accessions from the neighboring countries *i.e.*, India or Pakistan. Previous reports suggested that cowpea cultivars have been introduced into Oman from India and Pakistan. Some studies reported a trade of 500 years from southern part of Bombay and Oman (Allen, 1981) and between Oman and Baluchistan part from Pakistan (Grey, 1911). Future studies may be required to study relationship between accessions of cowpea from Oman and other countries because of the small sample size from Pakistan and India used in this study.

High genetic differentiation was noted among the accessions for morphological traits while low AFLP based genetic differentiation was noted between populations. High morphological differentiation indicated natural selection for adaptability to specific environment or farmer preference for specific ecotypes. The traits such as leaf shape, leaf color, leaf hairiness and leaf texture provide functional basis of variation to tolerate insect and drought tolerance.

Both methods showed that accessions “Mudhaibi”, “Liwa” and “Rustaq2” were genetically and morphologically diverse from rest of germplasm. These results showed that AFLP markers may reveal functional diversity. It has been noted earlier that AFLP markers were most suitable for assessing the genetic diversity in crop species germplasm and correlate it with phenotypic distance and heterosis (Agneta et al., 2014).

The study shows the presence of moderate to high levels of genetic diversity among populations of cowpea from various regions of Oman. These accessions should be conserved and may be used in future breeding programs for improving yield and disease resistance in the local and commercial cowpea cultivars.

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