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Cowpea (*Vigna unguiculata*) Landraces and Wild Relatives as Sources of Anthracnose Resistance: Implications for Breeding

Adenike O. Dada [1,2,3,*], Victor O. Dania [2], Olaniyi A. Oyatomi [3], Michael T. Abberton [3] and Alejandro Ortega-Beltran [3,*]

[1] Institute of Agricultural Research and Training, Obafemi Awolowo University, P.M.B. 5029, Moor Plantation, Ibadan 220288, Nigeria
[2] Department of Crop Protection and Environmental Biology, University of Ibadan, P.O. Box 4078, Ibadan 200132, Nigeria; vo.dania@mail.ui.edu.ng
[3] International Institute of Tropical Agriculture, IITA, P.M.B. 5320, Oyo Road, Ibadan 200001, Nigeria; o.oyatomi@cgiar.org (O.A.O.); m.abberton@cgiar.org (M.T.A.)
* Correspondence: aodada@iart.gov.ng or dada.adenike31@gmail.com (A.O.D.); a.beltran@cgiar.org (A.O.-B.)

Abstract

Cowpea (*Vigna unguiculata* (L.) Walp.) is a legume valued in several countries for its nutritional quality, low input requirements, and economic profitability. However, cowpea productivity is significantly constrained by anthracnose disease caused by *Colletotrichum* species. Enhancing host resistance through the introgression of resistance alleles from landraces and wild relatives offers a promising strategy for durable disease management. This study evaluated 20 cowpea landraces and 18 *V. unguiculata* ssp. *dekindtiana* accessions for resistance to anthracnose under natural field infection. The cultivars Ife Brown and *V. vexillata* line TVNu-428 were included as susceptible and resistant checks, respectively. The experiment was laid in an alpha lattice design (8 × 5) with three replications arranged. Anthracnose symptoms characterized by enlarged light-brown lesions surrounded by reddish-brown halos were produced on all the accessions and the symptoms progressed throughout the assessment period. Five *V. unguiculata* ssp. *dekindtiana* accessions (TVNu-113, TVNu-1506, TVNu-1222, TVNu-420, and TVNu-136) and three cowpea landrace accessions (TVu-14076, TVu-17556, and TVu-17567) were identified as promising sources of anthracnose resistance. These accessions represent valuable genetic resources for broadening the cowpea resistance gene pool and support the strategic utilization of wild cowpea germplasm in breeding programs.

Keywords: cowpea; landraces; wild relatives; anthracnose; sources of resistance

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1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important legume cultivated in several tropical and subtropical regions, particularly in sub-Saharan Africa (SSA) and South Asia [1,2]. The crop is highly valued for its adaptability to diverse agro-ecological conditions and its resilience to water and salt stress, enabling cultivation across a wide range of soil types and climatic conditions [3]. Cowpea plays a central role in food and nutrition security, especially within smallholder farming systems in SSA. Nearly all plant parts are consumed, including young leaves, immature pods, and dry beans, while crop residues serve as livestock feed [4,5].

Cowpea beans are rich in essential nutrients and bioactive compounds. They contain 20–30% protein and high amounts of thiamine, riboflavin, niacin, folates, iron, phosphorus,

calcium, magnesium, and zinc [6]. The beans contain 17 amino acids, including essential ones such as valine, lysine, leucine, and phenylalanine [1]. Cowpea beans are also low in fat and are gluten- and cholesterol-free, making them an attractive ingredient in food formulations. Additionally, their slowly digestible and resistant starch, phenolic acids, flavonoids, saponins, phytates, bioactive proteins, and peptides have chronic-disease-preventive properties [7,8].

Cowpea production is significantly constrained by biotic stresses, among which cowpea anthracnose disease (CAD) is of particular concern [9]. CAD is caused by the *Colletotrichum* species and reduces grain quality and market value [10]. Under conducive environmental conditions for pathogen establishment and proliferation, susceptible cultivars may become infected at any growth stage, from seedling emergence to harvest. In the case of CAD, several species of *Colletotrichum* have been identified globally. For example, *C. dematium* is often reported in South Africa [11]; *C. fructicola* in Iran [12]; and *C. lindemuthianum* and *C. destructivum* in India [13,14]. *Colletotrichum lindemuthianum* and *C. destructivum* were the only species named in Nigeria [10,15,16] until recently, when *C. truncatum* and *C. cliviicola* were reported as causal agents of CAD [17,18]. The complexity of the pathogen population underscores the importance of evaluating cowpea germplasm under field conditions where multiple *Colletotrichum* species may occur, providing more comprehensive insights into resistance sources with potential relevance across diverse agro-ecological and pathogen populations.

Management strategies for CAD include the application of synthetic fungicides, plant extracts, biocontrol agents, and the use of resistant cultivars [19–21]. However, reliance on synthetic fungicides is discouraged due to concerns about toxic residues and environmental contamination. In addition, use of fungicides may be cost-prohibitive for many smallholder farmers. Although plant-based extracts and biocontrol agents show promise, commercially viable, registered products have not been developed. Host plant resistance remains the most economical and environmentally sustainable approach in managing the disease. Nevertheless, variability within pathogen populations can lead to the breakdown of resistance in cultivated varieties. To overcome this, broadening the genetic base of cultivated cowpea is essential.

Crop wild relatives represent valuable reservoirs of genetic diversity for crop improvement [22]. They often possess traits that enable performance under harsh environmental conditions, and may harbor traits for resistance to pests and diseases [23,24]. More than 100 wild cowpea taxa have been documented [25], and many exhibit desirable agronomic characteristics and enhanced resistance to biotic stresses [26]. This includes the sub-species *dekindtiana*. Resistance genes from wild relatives have led to the development of improved cultivars in several crops. In the common bean (*Phaseolus vulgaris*), related *Phaseolus* species, including *P. coccineus*, have been identified as valuable sources of resistance to anthracnose caused by *C. lindemuthianum* [27].

The potential of cowpea landraces and cross-compatible wild relatives to confer resistance to cowpea diseases, particularly CAD, remains largely underexplored. Similarly, examination of cowpea landraces in their resistance to CAD has also received limited attention. It is currently unknown if genetic factors conferring stable, durable resistance to CAD exist among cowpea landraces and compatible wild *Vigna* species. Thus, it is necessary to investigate the resistance status of cowpea landraces and wild relatives to CAD. The objective of the current study was to screen cowpea landraces and cross-compatible *V. unguiculata* ssp. *dekindtiana* accessions for resistance to CAD with a view to identify resistant accession as potential CAD-resistant gene donors to improve the resistance of cowpea cultivars to this economically important disease.

2. Materials and Methods

2.1. Source of Cowpea Germplasm

Cowpea seeds were obtained from IITA-Ibadan Genebank. These comprised 20 accessions of cowpea landraces and 18 accessions of *V. unguiculata* ssp. *dekindtiana*. The cowpea variety Ife Brown was included as a susceptible check [17,28] and *V. vexillata* line TVNu 428 as resistant check (Table 1). TVNu 428 has been confirmed to show resistance to anthracnose in a pilot study.

Table 1. *Vigna* germplasm used to screen for resistance to anthracnose under natural infection.

Vigna unguiculata* ssp. *dekindtiana		**Cowpea Landrace**	
Accession ID	**Country**	**Accession ID**	**Country**
TVNu-108	South Africa	TVu-11595	Ghana
TVNu-109	Tanzania	TVu-12322	Unknown
TVNu-110	Tanzania	TVu-13968	Unknown
TVNu-113	Tanzania	TVu-14076	Central African Republic
TVNu-1222	Unknown	TVu-16908	Unknown
TVNu-136	Nigeria	TVu-17000	Nigeria
TVNu-1442	Mozambique	TVu-17517	Unknown
TVNu-145	Nigeria	TVu-17556	Unknown
TVNu-1506	Uganda	TVu-17567	Unknown
TVNu-1772	Namibia	TVu-17575	Unknown
TVNu-258	Chad	TVu-17581	Unknown
TVNu-297	Tanzania	TVu-17723	Unknown
TVNu-400	Nigeria	TVu-17731	Unknown
TVNu-413	Nigeria	TVu-17762	Unknown
TVNu-420	Nigeria	TVu-17784	Nigeria
TVNu-582	Niger	TVu-17870	Nigeria
TVNu-929	Unknown	TVu-17885	Nigeria
TVNu-941	Botswana	TVu-29	United States of America
-	-	TVu-3168	India
-	-	TVu-8850	Benin
Resistant Check		Susceptible Check	
TVNu-428	Botswana	Ife Brown	Nigeria

2.2. Experimental Site and Layout

The experiment was conducted at a research field in IITA-Ibadan during 2020 and 2021 and relied on CAD development under natural conditions. The field was selected based on its history of high CAD incidence, as cowpea grown in late 2019 and early 2020 exhibited severe infection. Studies conducted those years revealed that *C. cliviicola* [18] and *C. truncatum* was the predominant species in that field based on morphological characteristics and multi-locus DNA sequence comparisons. In a separate study, *C. truncatum* isolates from that field were sequenced and the sequences were deposited in NCBI GenBank (Accession nos. OR961038, OR965855 and OR965854; unpublished results).

The experiment was laid out in an alpha lattice design (8 × 5) with three replications. Each replication consisted of five incomplete blocks containing eight entries each, giving

a total of 40 plots per replication. Each plot consisted of a single 3 m row containing five plants spaced 75 cm apart within rows. Alleyways between plots and blocks were maintained at 1 m, while replications were separated by 2 m.

2.3. Sowing and Field Management

Three seeds of each *Vigna* accession were sown per hole and thinned to one plant per stand at two weeks after sowing. Plants were staked one month after planting to prevent clustering and inter-plant entanglement. Manual weeding was carried out regularly to maintain weed-free conditions throughout the experiment. No fungicides were applied in order to allow natural disease development.

2.4. Anthracnose Disease Severity Assessment

Anthracnose disease severity was assessed on each plot using the modified scale of [21] as follows: 1 = no visible lesions or only hypersensitive spots on the main stem and petioles of lower leaves; 2 = lesions extending to branches; 3 = advanced lesions on stems, petioles, branches, and veins on the abaxial leaf surfaces; 4 = severe infection with lesions on stems, petioles, branches, leaf veins, and peduncles; 5 = extensive lesions on stems, petioles, branches, and leaf veins, with spreading infection on peduncles and pods. Due to the relatively slow germination of *V. unguiculata* ssp. *dekindtiana* accessions, the first disease assessment was conducted four weeks after sowing. Subsequent assessments were carried out at fortnightly intervals until crop maturity. The area under the disease progress curve (AUDPC) is calculated according to the formula below [29]:

$$AUDPC = \sum_{i=1}^{n-1} \frac{(Y_{i+1}Y_i)}{2}(t_{i+1} - t_i) \quad (1)$$

where t_i represents the time (days after planting) at the ith observation, Y_i represents the disease severity at the ith observation, and n is the total number of observations. Based on the disease responses of the resistant (TVNu-428) and susceptible (Ife Brown) checks, accessions with mean AUDPC values < 10 were classified as resistant (R), those with values between 10 and 15 as moderately resistant (MR), and those with values > 15 as susceptible (S). These thresholds were selected to classify accessions with disease responses similar to the resistant check, the susceptible check, or an intermediate between the two.

2.5. Statistical Analysis

The mean severity values were subjected to analysis of variance (ANOVA) to evaluate the response of the accession to disease pressure in repeated trials during the 2020 and 2021 growing seasons. Prior to analysis, data were examined to ensure compliance with assumptions of normality and homogeneity of variance. Mean separation was carried out using New Duncan's Multiple Range Test (NDMRT) to show variability among accessions. In addition, accessions were classified into distinct categories of resistance and susceptibility based on their overall mean AUDPC values, and graphical representations were used to illustrate these groupings. All statistical analyses and visualizations were performed using the R software version 4.5.3.

3. Results

Typical symptoms of cowpea anthracnose disease (CAD) were observed on infected plants, characterized by enlarged light-brown lesions surrounded by reddish-brown halos (Figure 1). Lesions were first noticeable on the stems of most plants and subsequently progressed to other plant parts, including petioles, branches, veins, peduncles, and pods.



Figure 1. Anthracnose symptom expression in field conditions under natural infection in cowpea variety Ife Brown (left) and *Vigna unguiculata* ssp. *dekindtiana* TVNu-109.

Analysis of variance revealed significant differences among the cowpea landraces and *Vigna unguiculata* ssp. *dekindtiana* accessions for CAD severity across all assessment periods (5, 7, 9, 11, and 13 weeks after planting [WAP]; Table 2). The accession effect was highly significant ($p < 0.01$), with mean square values increasing from 1.43 at 5 WAP to a peak of 6.64 at 11 WAP, followed by a slight decline to 6.28 at 13 WAP.

Table 2. Mean square of cowpea anthracnose disease severity on selected cowpea landraces and *Vigna unguiculata* ssp. *dekindtiana* accessions in the field.

SV	DF	Mean Square of Anthracnose Severity				
		5WAP	7WAP	9WAP	11WAP	13WAP
Rep	2	0.73	1.97	4.00	4.55	1.32
Accession	39	1.43 **	2.31 **	4.01 **	6.64 **	6.28 **
Block(rep)	15	0.30	0.71	0.96	0.94	0.5
CV		20.73	13.74	13.07	10.32	16.15
R Square		0.76	0.78	0.75	0.80	0.53

SV = Source of Variation, DF = Degree of Freedom, WAP = Weeks After Planting, ** = significance at $p < 0.01$, CV = Coefficient of Variation.

Disease severity generally increased across most accessions as the season progressed (Tables 3 and 4). However, the resistant check, *Vigna vexillata* TVNu 428, consistently exhibited low severity scores throughout the experimental period, confirming its stable resistance under natural field conditions.

Table 3. Mean score of cowpea anthracnose disease severity on cowpea landraces in the field.

Accessions	5 WAP	7 WAP	9 WAP	11 WAP	13 WAP
Ifebrown (susceptible check)	2.3 c	3.7 c	5.0 d	7.0 c	8.0 abc
TVu-11595	2.7 b	4.0 b	5.0 d	7.3 b	8.2 ab
TVu-12322	2.0 d	3.0 e	5.0 d	7.0 c	8.0 abc
TVu-16908	2.3 c	3.3 d	4.3 f	5.7 h	6.5 hij
TVu-17000	2.3 c	3.3 d	4.3 f	5.7 h	6.5 hij
TVu-17517	2.3 c	4.0 b	5.3 c	7.3 b	8 abc
TVu-17556	1.3 g	2.7 g	4.0 g	5.3 i	6.3 jik
TVu-17567	1.7 e	2.7 g	4.0 g	5.7 h	6.5 hij

Table 3. *Cont.*

Accessions	5 WAP	7 WAP	9 WAP	11 WAP	13 WAP
TVu-17575	2.3 c	3.3 d	4.7 e	6.3 f	7.5 bcdef
TVu-17581	2.3 c	3.7 c	5.0 d	7.3 b	8.0 abc
TVu-17723	3.0 a	4.3 a	6.0 a	7.7 a	8.3 a
TVu-17731	2.0 d	3.3 d	4.7 e	6.3 f	7.5 bcdef
TVu-17762	2.7 b	4.0 b	5.3 c	7.3 b	8.2 ab
TVu-17784	3.0 a	4.0 b	5.7 b	7.7 a	8.3 a
TVu-17870	2.7 b	3.7 c	5.0 d	6.3 f	7.7 abcde
TVu-17885	2.7 b	4.0 b	5.7 b	7.7 a	8.3 a
TVu-29	2.0 d	3.3 d	5.3 c	7.3 b	8.0 abc
TVu-3168	3.0 a	4.3 a	5.7 b	7.3 b	8.2 ab
TVu-8850	2.7 b	3.7 c	4.7 e	7.3 b	8.2 ab
TVu-13968	2.3 c	3.3 d	4.7 e	7.0 c	8.0 abc
TVu-14076	1.3 g	2.3 h	3.7 h	4.7 j	5.3 l

Values with the same letters that are the same down the column are not significantly different (p value = 0.05). Means were separated using New Duncan's Multiple Range Test.

Table 4. Mean score of cowpea anthracnose disease severity on *Vigna unguiculata* ssp. *dekindtiana* accessions in the field.

Accessions	5 WAP	7 WAP	9 WAP	11 WAP	13 WAP
Ifebrown	2.3 c	3.7 c	5.0 d	7.0 c	8.0 abc
TVNu-108	2.3 c	3.7 c	4.7 e	7.0 c	8.0 abc
TVNu-109	1.7 e	3.3 d	5.0 d	7.0 c	7.8 abcd
TVNu-110	2.0 d	3.3 d	5.0 d	7.0 c	7.8 abcd
TVNu-113	1.0 h	2.0 i	2.7 j	4.3 k	5.7 kl
TVNu-1222	1.3 g	2.3 h	3.7 h	5.3 i	6.7 ghij
TVNu-136	1.3 g	2.7 g	4.0 g	5.7 h	6.5 hij
TVNu-1442	1.5 f	2.8 f	4.8 de	6.8 d	7.5 bcdef
TVNu-145	1.7 e	2.7 g	4.3 f	6.7 e	7.3 cdefg
TVNu-1506	1.0 h	2.0 i	3.2 i	5.3 i	6.2 jk
TVNu-1772	1.0 h	2.7 g	6.0 a	7.0 c	8.0 abc
TVNu-258	1.7 e	2.7 g	4.3 f	6.0 g	6.8 fghij
TVNu-297	2.0 d	3.0 e	4.0 g	6.0 g	7.2 efghi
TVNu-400	2.0 d	4.0 b	5.0 d	6.7 e	7.3 cdefg
TVNu-413	2.0 d	4.0 b	5.0 d	6.7 e	7.2 defgh
TVNu-420	2.0 d	3.0 e	4.0 g	5.3 i	6.2 jk
TVNu-428	0.7 i	1.2 j	1.3 k	2.0 l	2.7 m
TVNu-582	1.7 e	3.3 d	5.0 d	6.7 e	7.2 defgh
TVNu-929	2.0 d	3.7 c	5.3 c	7.0 c	7.8 abcd
TVNu-941	1.0 h	3.0 e	5.0 d	7.0 c	8.0 abc

Values with the same letters that are the same down the column are not significantly different (p value = 0.05). Means were separated using New Duncan's Multiple Range Test.

Further classification of the genotypes based on the area under the disease progress curve (AUDPC) indicated that the majority of cowpea landraces were susceptible (Figure 2). Notably, eight accessions (TVu-8850, TVu-29, TVu-17517, TVu-17762, TVu-3168, TVu-17885, TVu-17784, and TVu-17723) recorded AUDPC values higher than that of the susceptible check, Ife Brown, indicating a high level of susceptibility.

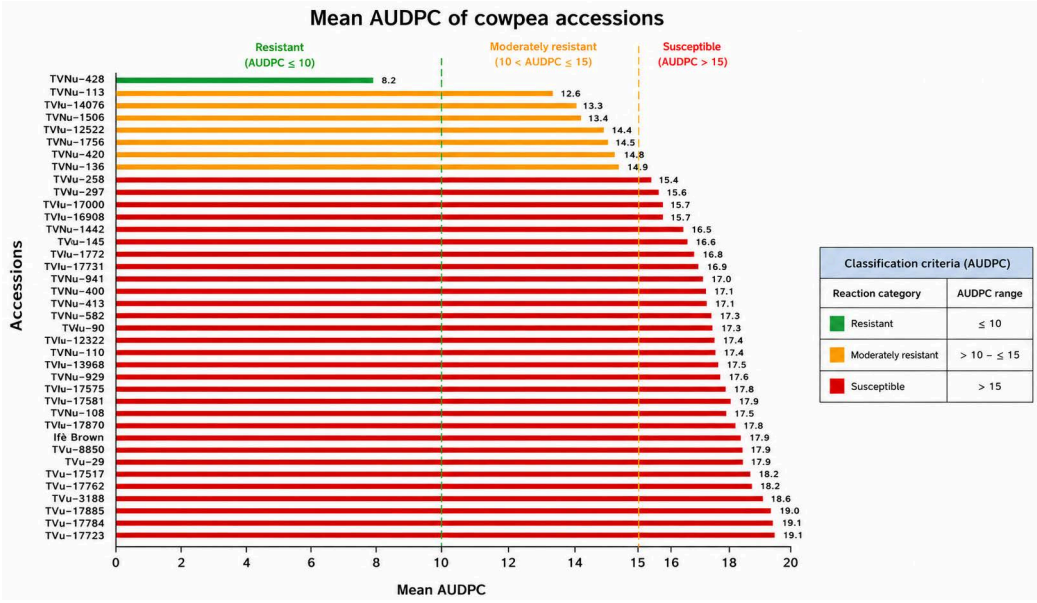


Figure 2. Mean AUDPC values of *Vigna* accessions and their resistance category.

4. Discussion

The study revealed the responses of varying accessions of cowpea landraces and *Vigna unguiculata* ssp *dekindtiana* to anthracnose infection caused by *Colletotrichum cliviicola* and *C. truncatum.* In Nigeria, cowpea anthracnose has traditionally been associated with *Colletotrichum lindemuthianum* and *C. destructivum*. However, recent reports have also implicated *C. cliviicola* and *C. truncatum* in anthracnose-like symptoms on cowpea, suggesting a potentially broader pathogen complex associated with the disease [17,18]. *C. truncatum* has been reported as a causal organism of anthracnose on diverse crops including tomato [30], swordbean [31], soybean [32], sorghum [33], chilli [34], and cabbage [35], while *C. cliviicola* (previously *C. cliviae*) has also been reported in multiple crops such as soybean [36], rubber [37], orchids [38], silver linden [39] and hybrid Pennisetum [40]. Although reports of these species on cowpea remain limited, their recovery and confirmed pathogenicity in the present study highlight the need for continued surveillance and evaluation of resistance sources against emerging *Colletotrichum* species.

Corresponding to the disease progression described by Latunde-Dada et al. [15], anthracnose symptoms were gradually produced on plant parts and their description were consistent with earlier reports [10,14,41]. While resistant lines to anthracnose caused by *C. cliviicola* have not yet been reported, *V. vexillata* is widely recognized for its resilience against several major biotic stresses, including resistance to cowpea mottle carmovirus (CPMoV) [42] and *Striga gesnerioides* [26], and have exhibited the claim in a previous trial on the field. Thus, the stable performance of this species in the present study reinforces its importance as a reservoir of resistance genes to major biotic stresses. Despite its demonstrated resilience, *V. vexillata* remains underutilized in cowpea improvement programs due to cross-incompatibility with cultivated cowpea. This limitation restricts direct gene transfer and necessitates screening of cross-compatible wild relatives like the *Vigna unguiculata* ssp *dekindtiana* accessions. The findings in this study showed increasing magnitude of mean severity which was observed till the 11th WAP, thus indicating intensified disease expression and greater differentiation among the accessions as the disease progressed. At this stage, genotypic variations in anthracnose resistance among the accessions became more pronounced and showed a similar pattern with the report of Pandey et al. [43] while screening mung bean accessions to anthracnose disease. The slight reduction observed in severity mean square at 13 WAP may reflect disease stabilization or the onset of plant

senescence, which can reduce further symptom differentiation. Replication effect was not significant at any assessment period, with relatively low mean square values ranging from 0.73 to 4.55. This indicates minimal environmental variation among replications and suggests that field conditions were reasonably uniform. Consequently, the significant differences observed among accessions can be attributed primarily to genetic factors rather than environmental heterogeneity. The coefficient of variation (CV) ranged from 10.32% at 11 WAP to 20.73% at 5 WAP, indicating acceptable experimental precision. The higher CV at 5 WAP likely reflects variability associated with early-stage disease establishment, when symptom expression was still developing and less uniform across plots. In contrast, the lower CV values at later stages, particularly at 11 WAP, suggest improved precision as disease symptoms became fully expressed and more uniformly distributed. The coefficient of determination (R^2) ranged from 0.53 to 0.80, indicating that the statistical model explained between 53% and 80% of the total variation in anthracnose severity. The highest R^2 value (0.80) was recorded at 11 WAP, corresponding with peak disease expression, when genotypic differences were most clearly manifested. The relatively lower R^2 at 13 WAP (0.53) may reflect increased environmental influence or reduced variability among accessions as the disease approached stabilization. Comparable findings were reported by Singh and Deepankar [44] in regression analyses of weather factors influencing mango anthracnose severity, where adjusted R^2 values ranged from 0.694 to 0.747. Collectively, these statistical indicators confirm the existence of considerable genetic variability for CAD resistance among the evaluated *Vigna* germplasm.

However, majority of cowpea landraces had AUDPC values exceeding that of the susceptible check, Ife Brown. This pattern suggests a relatively narrow resistance base among the evaluated landraces, increasing the risk of widespread disease outbreaks and potential resistance breakdown under pathogen variability. Only three landraces (TVu-14076, TVu-17556, and TVu-17567) and five accessions of *V. unguiculata* ssp. *dekindtiana* (TVNu-113, TVNu-1506, TVNu-1222, TVNu-420, and TVNu-136) were moderately resistant, highlighting limited but exploitable variation to introgress into improved backgrounds. Nevertheless, partial resistance is often considered more durable than monogenic resistance because it imposes lower selection pressure on pathogen populations, thereby contributing to long-term disease management [45]. The moderate resistance observed in these accessions suggests the presence of resistance loci that could be introgressed into elite but susceptible cultivars. Notably, *V. unguiculata* ssp. *dekindtiana* is cross-compatible with cultivated cowpea [26].

In general, the consistently significant genotype effect across all assessment periods confirms substantial genetic variation for CAD resistance within the evaluated *Vigna* germplasm. The identification of moderately resistant accessions, particularly within *V. unguiculata* ssp. *dekindtiana*, provides valuable genetic resources for breeding programs aimed at broadening the resistance base and enhancing durable CAD resistance in cultivated cowpea. Although the study was conducted under natural field infection rather than controlled inoculation, the experimental site is a known anthracnose hotspot with consistent disease occurrence. Nevertheless, environmental variation and uneven pathogen distribution under natural conditions may influence disease development and resistance expression. Therefore, further validation of the identified resistant accessions under controlled inoculation and across multiple environments would strengthen the reliability of the resistance ratings.

5. Conclusions

The current study demonstrated substantial genetic variability among evaluated *Vigna* germplasm in response to CAD. The significant genotype effects observed across

all assessment periods, together with progressive increases in disease severity over time, confirmed differential host responses and the presence of exploitable resistance within the studied materials. The resistant landraces (TVu-14076, TVu-17556, and TVu-17567) and *Vigna unguiculata* ssp. *dekindtiana* accessions (TVNu-113, TVNu-1506, TVNu-1222, TVNu-420, and TVNu-136) identified in this study represent promising sources of anthracnose resistance for cowpea improvement. These accessions could be exploited as donor parents in breeding programs aimed at introgressing durable resistance into high-yielding but susceptible cultivars. Furthermore, their utilization in resistance pyramiding and pre-breeding programs may contribute to the development of resilient cowpea varieties adapted to anthracnose-prone environments.

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Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

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Conflicts of Interest: The authors declare that there are no competing interests associated with this study.

Abbreviations

The following abbreviations are used in this manuscript:

CAD	Cowpea Anthracnose Disease
AUDPC	Area Under Disease Progress Curve
SSP	Sub-species

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