

Phenotypic Evaluation of Gene-Pyramided Cowpea Lines for Striga Resistance Across Nigerian Ecologies

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Abstract

Cowpea (*Vigna unguiculata* [L.] Walp.) is a critical protein source in sub-Saharan Africa, but its production is severely constrained by the parasitic weed *Striga gesnerioides*. This study evaluated two BC₃F₂ populations (140 lines). Population 1 was developed from {(IT90K-277-2 × B301) × (IT90K-277-2 × TVu-1272)} and Population 2 from {(IT90K-277-2 × TVu-16514) × (IT90K-277-2 × TVu-1272)}. Lines were screened against a homogeneous mix of *Striga* seeds from three endemic Nigerian environments (Kano, Borno, Jigawa) in a replicated pot trial in the screen house. Phenotypic evaluation revealed significant genetic variation ($p < 0.0001$) for both cowpea agronomic and *Striga* resistance parameters. Broad-sense heritability was high for *Striga* resistance parameters and yield components indicating their genetic control. Hierarchical clustering identified resistant BC₃F₂ lines in Cluster 1 that combined *Striga* resistance with high pod and seed weights, demonstrating no yield penalty. Conversely, Cluster 3 exhibited high susceptibility and poor yield. Strong negative correlations ($r < -0.8$) confirmed the negative impact of *Striga* on yield. Genetic architecture analysis supported a two-gene dominance model for *Striga* resistance BC₃F₂ populations, validated by chi-square tests ($\chi^2 = 2.34$; expected 1:1:1:1 ratio). These *Striga* resistance pyramided lines exhibit broad-spectrum resistance to *S. gesnerioides* from Kano, Borno and Jigawa, providing a validated breeding population for deploying durable, high-yielding cowpea varieties in *Striga*-endemic and major cowpea growing regions of Nigeria

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is a vital food and forage legume widely cultivated in subtropical and tropical regions, particularly in sub-Saharan Africa, where it serves as a key source of dietary protein (Timko and Singh, 2008). With an average protein content of 24% on a dry weight basis, cowpea is an affordable and nutritious staple for millions in West and Central Africa (Mafakheri *et al.*, 2017). Beyond its nutritional value, cowpea enhances soil fertility through intercropping systems and provides economic benefits as a cash crop (Carsky *et al.*, 1995; Noubissie *et al.*, 2010). Timko and Singh (2008), also report that cowpea has multiple advantages to farmers, including its ability to grow and produce high yields on poor, sandy soils unsuitable for the production of other crops, high rates of symbiotic nitrogen fixation, and lower fertilizer requirements.

According to Rivas *et al.* (2016) and Phillip *et al.* (2019), West Africa contributes approximately 95% to total global production of cowpea. Nigeria is the largest producer and consumer globally (Singh, 2002). According to FAOSTAT (2021), Nigeria had the highest production of cowpea with 3.63 million metric tons harvested from 4.7 million hectares, followed closely by Niger Republic, with 2.66 million metric tons harvested from 5.97 million hectares. In addition to Nigeria and Niger, other African countries with significant cowpea production include Burkina Faso, Cameroon, Ghana, Kenya, Uganda, and Tanzania (Nkomo, 2021). According to FAOSTAT (2021), Ghana, Tanzania, and Kenya produced 0.14 million metric tons from 0.2 million hectares, 0.13 metric tons from 0.15 million hectares, and 0.23 million metric tons from 0.25 hectares respectively.

Cowpea production faces significant constraints from both biotic and abiotic stressors. Among the biotic stresses, the parasitic weed *Striga gesnerioides* (Willd.) Vatke, is a major threat to productivity in the dry savannas of west and central Africa (WECA) (Kamara *et al.*, 2008; Omoigui *et al.*, 2018). *S. gesnerioides* is an obligate root hemiparasite that causes severe chlorosis, stunting, and even host plant death, leading to substantial yield losses ranging from 80% to 100% in susceptible cultivars (Olmstead *et al.*, 2001). Traditional control methods, including cultural practices and chemical treatments, have proven insufficient, making host plant resistance the most sustainable and economically viable solution (Singh, 2002). However, conventional breeding strategies relying on single-gene resistance are vulnerable to breakdown due to the emergence of diverse *Striga* races across West and Central Africa (Botanga and Timko, 2006).

The occurrence of different races of the parasite and the ability of *Striga* to overcome resistance call for deliberate stacking of resistance genes to effectively control the parasite and decrease the likelihood of resistance breakdown (Omoigui *et al.*, 2019). Recent advances in marker-assisted selection (MAS) offer a promising approach for accelerating the introgression of multiple resistance genes into elite cowpea varieties. Unlike conventional breeding, marker-assisted backcrossing (MABC) enables precise gene stacking in fewer generations, enhancing breeding efficiency (Boukar *et al.*, 2004; Mekonnen *et al.*, 2017). Furthermore, the identification of new sources of resistance to *S. gesnerioides* in TVu-16514 and TVu-1272 (Omoigui *et al.*, 2017), besides B301 which has mostly being the sole source of complete resistance to Striga across several countries (Singh and Emechebe 1990), provides an opportunity to broaden the genetic base of Striga resistance in cowpea.

This study was therefore designed to evaluate the performance of gene-pyramided elite cowpea lines against *S. gesnerioides* across different striga endemic environments in Nigeria. By integrating resistance genes from diverse genetic sources into farmer-preferred cultivars, this study seeks to enhance durable resistance and sustainable cowpea production in Striga-infested regions.

Materials and Methods

Experimental Materials, Design and Location Two populations of BC₃F₂ cowpea lines, pyramided with Striga resistance genes from cowpea genotypes B301, Tvu-16514, and Tvu-1272, were used as the experimental population to evaluate for resistance to *Striga gesnerioides*.

The characteristics and descriptions of the four (4) parental lines used in this study to generate the two BC₃F₂ populations are presented in Table 1.

Table 1
Parental Material used in the Study

Cowpea genotypes	Origin	Gene	Response to <i>Striga</i>	SG Races
IT90K-277-2	Improved variety from IITA Breeding Program Nigeria	n/a	S	n/a
Tvu-16514	Landrace from Burkina Faso	Rsg2	R	SG2
Tvu-1272	Landrace from Nigeria	Rsg1	R	SG1
B301	Landrace from Botswana	Rsg3	R	SG3
Key: R = Resistant Cultivar S = Susceptible Cultivar, SG = Races of <i>Striga gesnerioides</i> ,				

Population 1 was generated from {(IT90K-277-2 x B301) x (IT90K-277-2 x Tvu-1272)}, while Population 2 was generated from {(IT90K-277-2 x TVu-16514) x (IT90K-277-2 x Tvu-1272)}.

Simultaneous Pyramiding of Resistance Genes and Population Development

Simultaneous scheme of gene pyramiding via backcrossing for transfer of Striga resistance from donor parents, and recovery of recurrent parent genome was conducted. Crossing was done by emaculation as described by Myers (1996).

The susceptible recurrent parent- IT90K-277-2, was crossed with B301 as a Striga resistant donor parent to obtain an F₁. Similarly, IT90K-277-2 was crossed with a Tvu-1272 as another Striga resistant donor parent to obtain F₁ as well. Then the two F₁s were crossed to together to generate F₁ pyramided with Striga resistance. Next the F₁ {(IT90K-277-2

x B301) x (IT90K-277-2 x Tvu-1272)} was backcrossed with the recurrent parent IT90K-277-2, up to BC₃F₁. At each backcross, SSR1 marker (Table 2), known to segregate with Striga resistance in cowpea (Omoigui *et al.*, 2017), was used in foreground selection for Striga resistance genes from the donor parents' genomes, to select individual plants with the desired resistance genes. Only the F₁ plants carrying the desired Striga resistance genes as tracked by the SSR1 marker were crossed with the recurrent parent, IT90K-277-2 to generate a backcross.

In the same manner, IT90K-277-2, was crossed with a donor resistant parent- TVu-16514 to obtain F₁. IT90K-277-2 was again crossed with a donor resistant parent-Tvu-1272 to obtain F₁. Then the two F₁s were crossed to together to generate F₁ pyramided with Striga resistance. Next the F₁ {(IT90K-277-2 x TVu-16514) x (IT90K-277-2 x Tvu-1272)} was backcrossed with the recurrent parent IT90K-277-2, up to BC₃F₁. At each backcross, SSR1 marker was used in foreground selection for Striga resistance genes from the donor parents' genomes, to select individual plants with the desired resistance genes. Only the F₁ plants carrying the desired Striga resistance genes as tracked by the SSR1 marker were crossed with the recurrent parent, IT90K-277-2 to generate a backcross.

Using this simultaneous gene pyramiding approach two populations (Population 1 and population 2) were generated in this study. The BC₃F₁ of each of this populations were then selfed to generate the respective BC₃F₂. Figures 1 and 2 shows the backcrossing scheme that was used to develop cowpea *Striga* resistance pyramided lines in this study.

Table 2
Description of the SSR1 Marker Associated with *Striga gesneriodes* Resistance in Cowpea

Marker Name	Marker Sequence	Trait associated with
SSR1 Forward Primer	CCT AAG CTT TTC TCC AAC T CAA GAA GGA GGA GGC	Striga gesneriodes Resistance
SSR1 Reverse Primer	CAA GAA GGA GGC GGC GAA GAC T CAA GAA GGA GGA GGC	Striga gesneriodes Resistance

Striga seeds used in this study were collected from three different environments in Nigeria- Kano state (Migibri) (Latitude: 12.0022° N, Longitude: 8.5920° E), Borno State (Malammodori) (Latitude: 12.3333° N, Longitude: 10.4167° E) and Jigawa State (Sule Tankaka) (Latitude: 11.7574° N, Longitude: 9.3377° E), which are Striga endemic areas as well as major cowpea production areas in Nigeria.

Evaluation of BC₃F₂ 140 lines of the BC₃F₂ populations (70 lines from each population) were screened for *Striga gesneriodes* resistance using a pot culture technique as described by Omoigui et al (2017). Pots were filled with a 2:1 mixture of topsoil and sand and inoculated with 5,000 Striga seeds.

First 5g (measured using a small weighing boat) each of the Striga seeds collected from Kano, Jigawa and Borno were homogeneously mixed. This approach was to ensure that BC₃F₂ that will be selected, had resistance to Striga from the three environments.

Then the number of Striga seeds in the mixture was determined using the method of Ugbaa et al (2020). One thousand (1000) Striga seeds were counted from the Striga seed mixture using a compound microscope (Brunel Microscope 0723219. X400 magnification) and weighed with a digital sensitive balance (Denver Instruments TP-3002).

The weight of five thousand (5000) Striga seeds in grams was then extrapolated as shown below.

Weight of 1000 seeds = 0.125g

Therefore, weight of 5000 seeds = $0.125\text{g} \times 5 = 0.625\text{g}$

The pot culture inoculated with the mixed Striga seeds, was preconditioned for seven days to enhance Striga seed germination. The experiment was arranged in a Completely Randomized Design replicated three times.

Data Collection The following observations were made on Striga parameters: Day to first Striga emergence, Striga count at 42 Days after planting, Striga count at 49 Days after planting, Striga count at 56 Days after planting, Number of Striga attached, Striga Haustorium attached, Striga Height at 56 Days after planting (cm) and Striga dry weight (g)

After the harvesting of seeds from individual cowpea plants, the soil was washed off the cowpea plant roots after submerging each pot in a 20L bucket of water for 5 minutes. The cowpea plants were carefully washed free from any remaining soil and examined closely for Striga haustorium attachment. Plants allowing attachment, haustorium development, and emergence of Striga shoots were classified as susceptible, while those without any attachment and free of infection were categorized as resistant.

The following observations were also made on cowpea plant parameters: Day to first flowering, Plant height at 3 weeks after planting (cm), Plant shoot dry weight (g), Plant root dry weight (g), Number of Pods per plant, 100 seed weight (g)

The above data was collected from the 140 individuals of the BC_3F_2 and their parents.

Data Analysis The phenotypic data of cowpea plant and Striga parameters of the BC_3F_2 were subjected to analysis of variance (ANOVA) using GenStat software (17th Edition, VSN International Ltd.). Significant differences among lines were determined using Duncan's Multiple Range Test (DMRT).

The phenotypic data were further analyzed through cluster analysis using the ClustVis web tool (Metsalu and Vilo, 2015), which implements the *pcaMethods* R package and in this work Euclidean distance with Ward's linkage method were applied for the analysis.

Chi-square test was performed to ascertain the goodness-of-fit of the observed segregation pattern for Striga resistance in the BC_3F_2 populations, to the expected phenotypic ratio for backcross populations using the following formula.

$$X^2 = \sum \frac{(O - E)^2}{E}$$

Where:

X^2 = chi-square test statistic

Σ = summation operator, meaning "take the sum of"

O = observed value

E = expected value

The assumptions for the Chi-square test for goodness-of-fit in this study are outlined as follows:

1. The two genes responsible for resistance are assorting independently during gamete formation, leading to the expected 1:1:1:1 ratio of genotypes (Resistant = RrSs , Rrss , rrSs , Susceptible = rrss) in the backcross population.
2. The two genes are not linked

Table 3: Gene Action Based on Graph form, Skewness and Kurtosis

Normality test	Graph form	Gene action/number of genes
Skewness = 0	Normal distribution	Additive gene action
Skewness < 0	Abnormal distribution	Additive gene action with the effect of duplicate epistasis
Skewness > 0	Abnormal distribution	Additive gene action with the effect of complementary epistasis
Kurtosis = 3	Mesokurtic	
Kurtosis < 3	Platykurtic	Many genes (polygenic)
Kurtosis > 3	Leptokurtic	Few genes

Source: Dasriani *et al.* (2020)

Results and Discussion

Phenotypic Screening of Striga Resistance Pyramided BC₃F₂ Lines Table 4 presents the results of analysis of variance (ANOVA) and heritability estimates for the evaluation of BC₃F₂ Striga resistance pyramided lines of Population 1 and Population 2 for various traits in this study. Mean square estimates were highly significant ($p < 0.0001$) for Genotype, Population and their interaction for cowpea plant parameters and Striga parameters evaluated, indicating strong genetic and influence on trait expression.

In addition, the highly significant mean squares for genotype and population, for the evaluated parameters indicates notable variation for agronomic traits and Striga resistance among the population. The results suggested that genotypes performed differently across the BC₃F₂ populations in response to inherent genetic variations for agronomic traits and Striga resistance. Additionally, the notable variation indicates that different resistance genes from the 3 donor parents used in the generation of the BC₃F₂ populations were combined in various lines within the populations. The highly significant genotype $\times$ population interaction also suggests that some lines may have unique combinations of resistance genes and highlights the importance of multi-environment testing. For instance, a resistance gene from a particular donor parent may be more effective against Striga from either Kano, Jigawa or Borno environment, suggesting a differential in the striga population from these environments. The resistance response of the BC₃F₂ lines shows that the pyramided lines have durable, multi-environment Striga resistance. This shows the possibility for selection of BC₃F₂ lines with superior agronomic performance and combined resistance to Striga found in Kano, Borno and Jigawa states respectively. Attamah *et al.* (2024) who pyramided aphid and striga resistance in cowpea, demonstrated that pyramiding genes from multiple donors led to enhanced and more stable resistance.

Broad-sense heritability (h^2) ranged from 0.61 to 0.80, suggesting moderate to high genetic control. The moderate to high heritability values (0.61–0.80) for all traits indicate significant genetic control. This aligns with findings from Boukar *et al.* (2019) in their review of cowpea breeding progress, where they reported similar heritability ranges for various agronomic traits. Yield components such as 100-seed weight ($h^2 = 0.80$) and seed weight per plant ($h^2 = 0.72$) also demonstrated strong genetic determination, supporting their amenability to selection in the breeding programs. The high heritability for yield components is particularly promising for this breeding program, opening up the

possibility for selecting high yielding elite lines with broad based Striga resistant lines in this study. Huynh et al. (2018) found comparable heritability estimates for flowering time ($h^2 = 0.66$ in this study), confirming the genetic control of this trait.

Plant architecture and biomass (plant height at 3 weeks, shoot dry weight, and root dry weight) and yield components (pods per plant, pod weight, seed weight, and 100-seed weight) both shows high heritability (0.75–0.77) and (0.72–0.80) respectively, agrees with findings from Aliyu and Makinde (2016), who reported high heritability for yield components in cowpea, suggesting good potential for selection and improvement.

Striga resistance traits (days to first emergence, number attached, haustorium attached) have high heritability ($h^2 = 0.73–0.76$) suggests strong genetic control of Striga resistance. This aligns with Omoigui et al. (2017), who found significant genetic variation for Striga resistance in cowpea. The increasing heritability of Striga counts over time (0.61 at 42 to 0.76 at 56 days after planting) is interesting and may indicate that resistance mechanisms become more pronounced as plants mature. Delayed emergence can reduce the competitive advantage of Striga, allowing the cowpea plants to establish themselves better before the parasite attaches. This aligns with previous studies indicating that resistant cowpea varieties can delay Striga emergence and reduce its attachment rates, thereby mitigating yield losses associated with infestation (Lane et al., 2003; Boukar et al., 2016).

Additionally, the variation among the Striga resistance pyramided lines for parameters such as Striga counts at earlier stages (42 and 49 days after planting), as well as for parameters like Striga height and dry weight indicates that some lines may be more effective at suppressing Striga growth, which is crucial since Striga can significantly affect host plant vigor and yield (Asare et al., 2020). The ability to restrict Striga haustorial attachment and growth is essential, as it has been documented that *S. gesnerioides* can cause damage even before emergence substantial yield reductions in susceptible cowpea varieties.

The ability to restrict Striga haustorial attachment and growth is indeed crucial, as *Striga gesnerioides* causes significant damage to cowpea, often before the parasite even emerges from the soil (Singh and Emechebe, 1990; Ayeni et al., 2017). According to Parker, (2012), this damage occurs through the parasitic attachment of Striga to the cowpea roots, where it siphons off essential nutrients and water from the host plant, leading to stunted growth, chlorosis, and yield loss and extreme cases even death of the host plant. Studies have shown that yield reductions in susceptible cowpea varieties can be as high as 80–100% under severe infestations (Singh and Emechebe, 1990).

The damaging effects of Striga before emergence emphasize the need for cowpea varieties that can restrict haustorial attachment at the root level. This underscores the significance of the Striga resistance pyramided lines carrying broad resistance to Striga from different environments in the major cowpea growing region of Nigeria, which demonstrated no haustorial attachment in this study. These lines offer an opportunity for the development of cowpea materials that offer durable genetic resistance via a protective mechanism that arrests Striga growth at this critical early stage of the crops field establishment. By exploiting the genetic resources developed in this study, breeders can develop cowpea varieties with enhanced resistance to Striga, thereby protecting yields and promoting food security in the cowpea growing regions susceptible to *Striga gesnerioides* infestation. Therefore, cowpea breeding efforts should continue to prioritize resistance to haustorial attachment as a primary strategy for controlling Striga infestations, particularly in regions like Kano, Borno, and Jigawa, where Striga is endemic and capable of causing devastating yield reductions.

Clustering of BC₃F₂ Genotypes and Character Associations

Figure 3 presents the results of hierarchical clustering and heatmap analysis of the BC₃F₂ cowpea population (combined population 1, 2 and parental genotypes) based on cowpea plant and Striga parameters (19 parameters) evaluated in this study. The genotype dendrogram in Fig. 3 (dendrogram on top of the figure) revealed three distinct phenotypic clusters of the BC₃F₂ individuals, each with unique parameters. The clustering pattern indicates distinct genetic groups within the population, in terms of cowpea plant and Striga parameters evaluated, with cluster I appearing to be the most distant. This clustering pattern aligns with findings from Xiong et al. (2016), who observed similar clustering patterns in cowpea genotypes using SNP markers. They found that cowpea accessions often grouped based on geographical origin or breeding history.

Cluster 1 (top tier of the heatmap) is composed BC₃F₂ individuals exhibiting low Striga infestation (blue coloration) for traits like Striga dry weight, haustorium attachment, and Striga counts at 42–56 days after planting, coupled with yield (red coloration) related traits like pod weight, seed weight, and seeds per plant. This indicates that these BC₃F₂ individuals successfully combine resistance with productivity, aligning with the goal of introgressing resistance alleles without yield penalties. The unique BC₃F₂ individuals in the cluster include Lines 03, 110, 83, 108, 116, 134, 119, 134, 119, 138, 115, 136, 131, 123, 135, 66, 90, 67, 112, 121, 129, 118, 27, 139, 101, 69, 113, 127 and 2. These lines are good candidates for advancement for the development of new cultivars or as elite parental lines for future crosses.

In contrast, Cluster 3 (bottom tier) is made up of susceptible BC₃F₂ individuals with high Striga parameter values (red) and poor performance for cowpea plant parameters (blue). This suggests that Striga parasitism severely compromises yield, consistent with findings by Larweh *et al.* (2019) in cowpea. The figure also shows that Cluster 2 is the largest and most heterogeneous group of BC₃F₂ individuals. It is made up of BC₃F₂ individuals with intermediate or mixed phenotypes, implying polygenic control of these traits, this observation aligns with that of Batieno et al. (2016) who described how the BC₃F₂ population derived from marker-assisted backcrossing exhibited variation in phenotypic expression, particularly for traits like yield and Striga resistance.

The hierarchical clustering heatmap (Fig. 3) also show dendrogram of Striga parameters and Cowpea plant parameters (top dendrogram) and provide a visual representation of trait relationships among the evaluated parameters, which are validated quantitatively by the correlation matrix (Fig. 4). The correlation matrix presents a vertical numerical scale of correlation coefficients ranging from - 1 (perfect negative correlation) to + 1 (perfect positive correlation), corresponding to the colour patterns in the Fig. 4, and a validation for the colour patterns the heat map (Fig. 1)

Figure 3 shows that, Striga counts at various 42, 49, and 56 DAP, Striga dry weight, Striga height at 36 DAP, Striga haustorium attachment, and Striga reaction status clustered tightly together. The correlation matrix (Fig. 4) validates this result, showing strong positive correlations (light red to dark red squares, $r = 0.4$ to 0.8) among Striga count at 42, 49 and 56 DAP, number of Striga attached, Striga haustorium attached, Striga height at 56 DAP, and Striga dry weight. This strong correlation indicates that these traits are a measure of cowpea plant susceptibility/resistance to Striga parasitism. This suggests that for future phenotyping, a subset of these traits, such as Striga count at an 42 DAP, could serve as a reliable measure for overall infestation. This could streamline phenotyping efforts and help to accelerate breeding cycles.

Figure 3 also show that Pod weight, Seed weight, 100 seed weight, Number of seeds per pod, and Number of seeds per plant also clustered tightly together. Similarly, the correlation matrix (Fig. 4) validated this result with strong positive correlations (dark red squares) among these yield related cowpea plant parameters. These traits are critical for assessing the productivity of the BC₃F₂. Aliyu et al. (2016) reported similar correlations in cowpea yield components, supporting the interrelationship of these traits. The strong positive correlations among these yield components are expected, and are desirable as improvement in one component can lead to improvements in other

correlated components. The correlation of yield components provides an opportunity for the indirect selection for yield using yield components as proxy in subsequent generations as the population is advanced for cultivar development. Boukar et al. (2019) emphasized the importance of considering these correlations in breeding programs for yield improvement.

Notably, Plant shoot dry weight and Plant root dry weight were also strongly associated with the yield cluster in both Fig. 3 and Fig. 4. The correlation matrix (Fig. 4) would show strong positive correlations between Root dry weight and the yield components. This underscores the critical role of a good root system in determining host plant performance under *Striga* parasitism. A good root system is important because it facilitates efficient uptake of water and nutrients, which are essential for plant growth and yield. Under *Striga* infestation, the parasite competes for these vital resources (Press, 1995). A more vigorous root system might be better able to compensate for resources lost to the parasite thereby contributing to tolerance to the parasite. Additionally, the hyperactive resistance mechanism of cowpea resistance to the *Striga* parasite is expressed in the root system to impede parasite attachment or development, thereby contributing to tolerance or resistance (Muranaka et al 2011). This implies that root traits, often overlooked in above-ground phenotyping, should be considered as important indirect selection targets for both *Striga* tolerance and overall yield improvement.

Figure 3 showed a striking negative association between the *Striga* infestation parameter cluster and the yield component parameter cluster. The figure showed that genotypes exhibiting high values for *Striga* traits (red coloration) showed low values for yield traits (blue coloration). This result is validated by the correlation matrix in Fig. 4, showing strong negative correlations ($r < -0.8$, dark blue squares) between all *Striga* parameters and all yield components.

The negative correlation between *striga* parameters and yield components demonstrates the severe detrimental impact of *Striga* parasitism on cowpea yields. It underscores the yield penalty associated with susceptibility, where host resources are diverted to the parasite at the expense of the host's own growth and reproduction. While resistance is critical to limit these losses, previous studies have reported potential trade-offs. For instance, Kamara et al. (2008) observed trade-offs between *Striga* resistance and yield in some cowpea lines, and Timko and Singh (2008), noted that resistance mechanisms often come at a metabolic cost, potentially reducing yield. On the other hand, Boukar et al. (2019) reported on cowpea lines that combined high levels of *Striga* resistance with good yield potential, suggesting that this trade-off is not universal and can be overcome through targeted breeding.

Studies in other crops, including cowpea, have also reported strong negative correlations between grain yield and *Striga* counts have been reported. Omoigui et al. (2012) reported a strong negative correlation was observed between grain yield and *Striga* counts in cowpea. In a similar trend Halidu et al. (2015) also reported negative correlation between grain yield and *striga* infestation. This underscores the genetic worth of the BC₃F₂ lines with broad resistance to *Striga* for the development of *striga* resistant elite cultivars for food security in Nigeria. Cowpea cultivars with broad *striga* resistance offer a valuable tool for climate resilience, as drier conditions and declining soil fertility exacerbated by climate change increase *striga* infestations.

Table 4
Mean square estimate, P values and Heritability for Striga and Plant Parameter from Reaction of BC₃F₂ segregating population 1 and 2 to striga infestation

Parameter	Genotype	Population	Genotype x Population	Genotype	Population	Genotype x population	Heritability
	Mean square estimates					P value	
Days to first flower	8444.47	1089.2	12293.47	< 0.0001	< 0.0001	< 0.0001	0.66
Plan height @ 3 weeks DAP	33.55	262.01	30.86	< 0.0001	< 0.0001	< 0.0001	0.77
Days to first striga emergency	538.55	1537.33	344.96	< 0.0001	< 0.0001	< 0.0001	0.76
Striga count @ 42 DAP	0.36	0.04	0.37	< 0.0001	< 0.0001	< 0.0001	0.61
Striga count @ 49 DAP	5.98	1.81	5.79	< 0.0001	< 0.0001	< 0.0001	0.69
Striga count @ 66 DAP	16.63	49.32	12.43	< 0.0001	< 0.0001	< 0.0001	0.76
Number of striga attached	15.77	50.97	12.35	< 0.0001	< 0.0001	< 0.0001	0.75
Striga haustorium attached	2.42	7.89	2.08	< 0.0001	< 0.0001	< 0.0001	0.73
Striga height @ 56 DAP	15.03	5.84	10.81	< 0.0001	< 0.0001	< 0.0001	0.69
Striga dry weight	1.18	4.58	1.21	< 0.0001	< 0.0001	< 0.0001	0.71
Plant shoots dry weight (g)	24.59	438.43	22.29	< 0.0001	< 0.0001	< 0.0001	0.77
Plant root dry weight (g)	11.98	58.5	11.92	< 0.0001	< 0.0001	< 0.0001	0.75
Pods per plant	1.79	16.3	1.71	< 0.0001	< 0.0001	< 0.0001	0.76
Pod weights (g)	7.75	33.23	5.94	< 0.0001	< 0.0001	< 0.0001	0.80
Seed weight per plant	1.32	15.84	1.52	< 0.0001	< 0.0001	< 0.0001	0.72
100 seed weight (g)	102.24	639.42	78.78	< 0.0001	< 0.0001	< 0.0001	0.80
Number of seeds per plant	95.62	1436.25	119.22	< 0.0001	< 0.0001	< 0.0001	0.71
Number of seeds per pod	25.63	451.02	34.56	< 0.0001	< 0.0001	< 0.0001	0.69

Genetic Architecture and Inheritance of Striga Resistance

Table 7 presents the observed and expected phenotypic classes in the two BC₃F₂ populations along with the calculated chi-square (χ^2) values. The chi-square values for both population 1 (Pop-1) and population 2 (Pop-2) were calculated as 2.34, which is significantly lower than the critical value of 7.815 at the 5% level of significance (df = 3). The non-significant chi-square value indicates that the observed segregation ratios do not deviate significantly from the expected 1:1:1:1 ratio, suggesting that the populations are segregating for two independent genes controlling resistance and susceptibility to *Striga gesnerioides*.

Table 7
Chi-square Goodness-of-Fit to Expected Genetic ratio for Backcross with two Genes

Generation	N	Observed				Expected				Genetic ratio	Calculated χ^2 value	Critical χ^2 value
		R		S		R		S				
		RrSs	Rrss	rrSs	rrss	RrSs	Rrss	rrSs	Rrss			
Pop1 (BC3F2)	70	20	19	19	12	17.5	17.5	17.5	17.5	1:1:1:1	2.34*	7.815
Pop-2 (BC3F2)	70	19	20	19	12	17.5	17.5	17.5	17.5	1:1:1:1	2.34*	7.815
*= significant calculated χ^2 value, indicating “goodness-of-fit” to Genetic ratio at 95% level of significance; N = Number of lines evaluated; R = Number resistant; S = Number susceptible; Resistant genotypes = RrSs, Rrss, rrSs, Susceptible genotypes = rrss												

This result is consistent with the pyramiding of dominant resistance genes from two donor parents in each of the BC₃F₂ populations. This aligns with previous studies by Singh and Emechebe (1990) and Atokple *et al.* (1993), who reported single-gene dominance for Striga resistance in cowpea. The polygenic nature of Striga resistance in each of the BC₃F₂ populations, as indicated by the distribution of resistance traits, underscores the importance of pyramiding multiple resistance genes to achieve durable resistance (Boukar et al., 2016).

Conclusion

The phenotypic evaluation of BC₃F₂ cowpea lines pyramided for *Striga gesnerioides* resistance revealed significant variation for Striga resistance parameters and cowpea plant parameters under striga infestation. The observed variation and significant genotype × population interaction showed the successful introgression and segregation of resistance alleles from the donor parents, leading to differential expression of both agronomic and Striga resistance traits. The moderate to high broad-sense heritability estimates across Striga resistance parameters and yield-related parameters indicating the strong genetic control of these traits and support their utility for effective selection in early-generation breeding.

The hierarchical clustering and heatmap analyses identified a subset of BC₃F₂ lines with combined high resistance and strong agronomic performance, highlighting the possibility of breaking the resistance-yield trade-off often reported in cowpea breeding. The negative correlation between *Striga* parameters and yield components underscores the impact of *Striga gesnerioides* on susceptible cowpea genotypes, and validates the need for effective resistance breeding strategies.

Genetic architecture analysis based chi-square test, pointed to a two-gene model of resistance segregating independently in the BC₃F₂ populations evaluated in this study. This shows the dominance nature of *Striga* resistance in cowpea mediated by major genes.

These results demonstrate the potential of the pyramided BC₃F₂ lines to serve as a valuable genetic resource for breeding cowpea cultivars with durable and broad-spectrum *Striga* resistance. The identification of lines that combine high resistance with yield stability under infestation, will be particularly in useful in the *Striga*-endemic zones of Kano, Borno, and Jigawa.

Declarations

Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

Isaiah A. Gabriel: Conceptualisation (lead); Writing – original draft (lead); Formal analysis (lead); Writing – review and editing (equal); Methodology (lead); Data curation (lead); Visualisation (lead). Lucky O. Omoigui: Supervision (lead); Validation (lead); Writing – review and editing (equal).

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Table Information

Table numbers 5 and 6 are not used in this version.

Figures

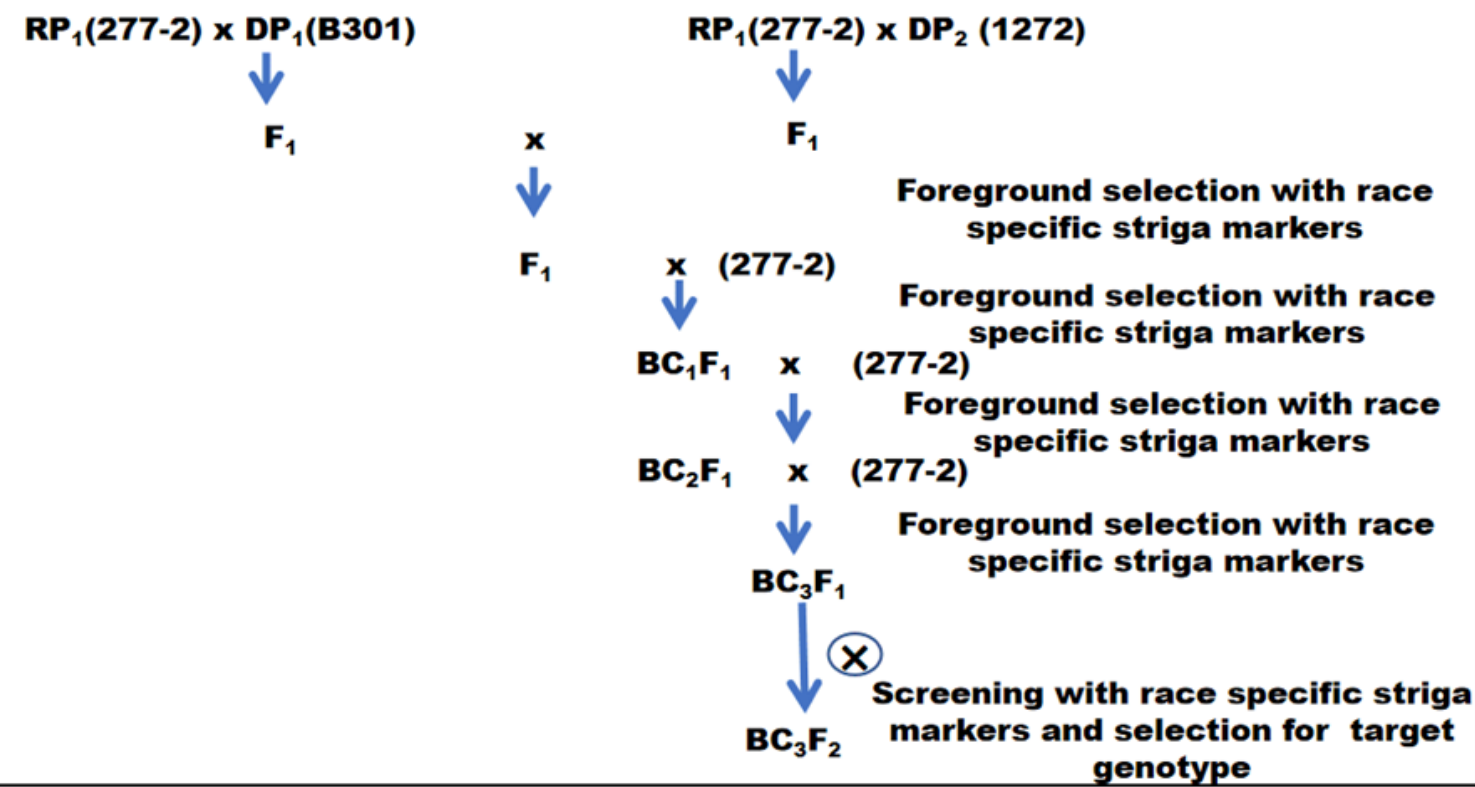


Figure 1

Generation of population 1 for the study. Gene pyramiding of *Striga* resistance from different sources using marker-assisted backcrossing

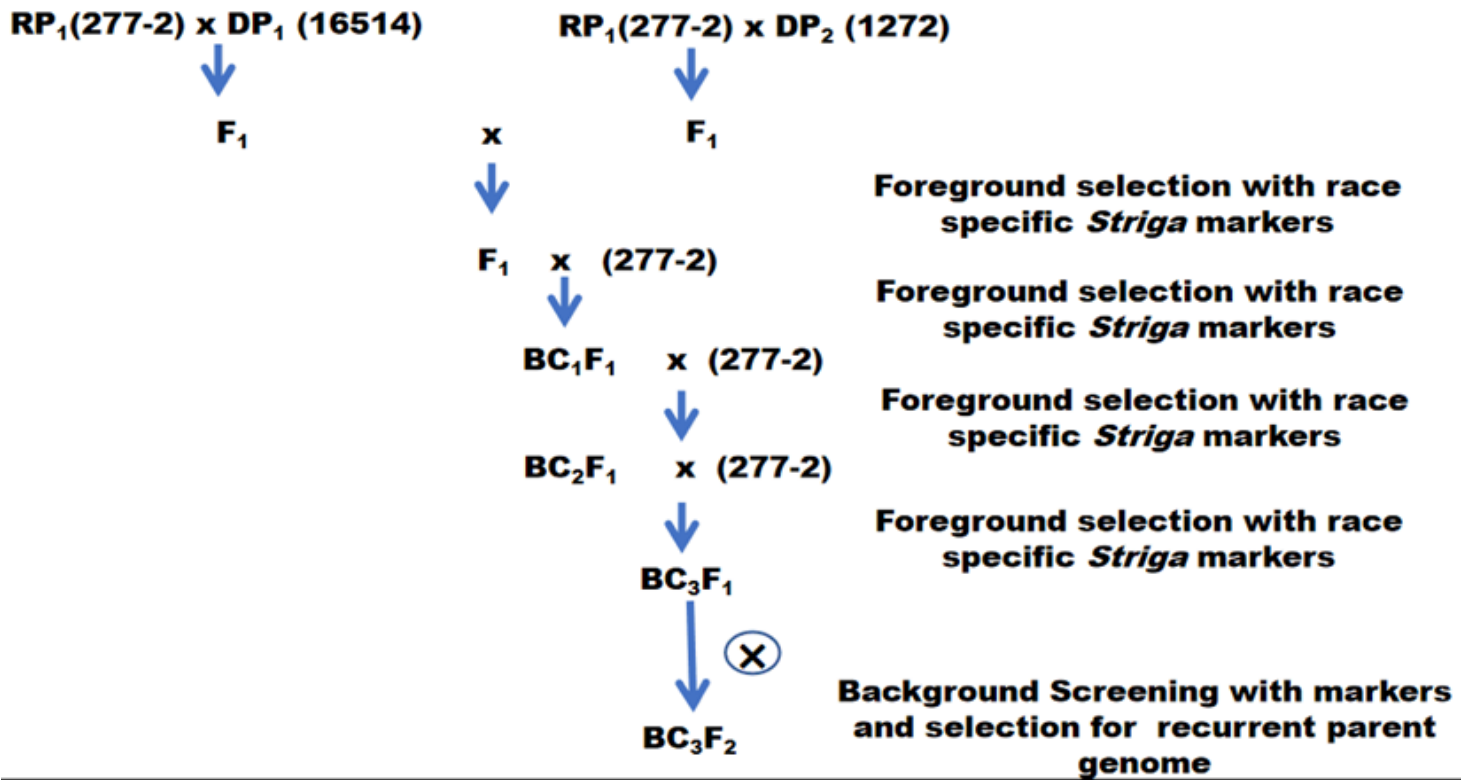


Figure 2

Generation of population 2 for the study. Gene pyramiding of *Striga* resistance from different sources using marker-assisted backcrossing

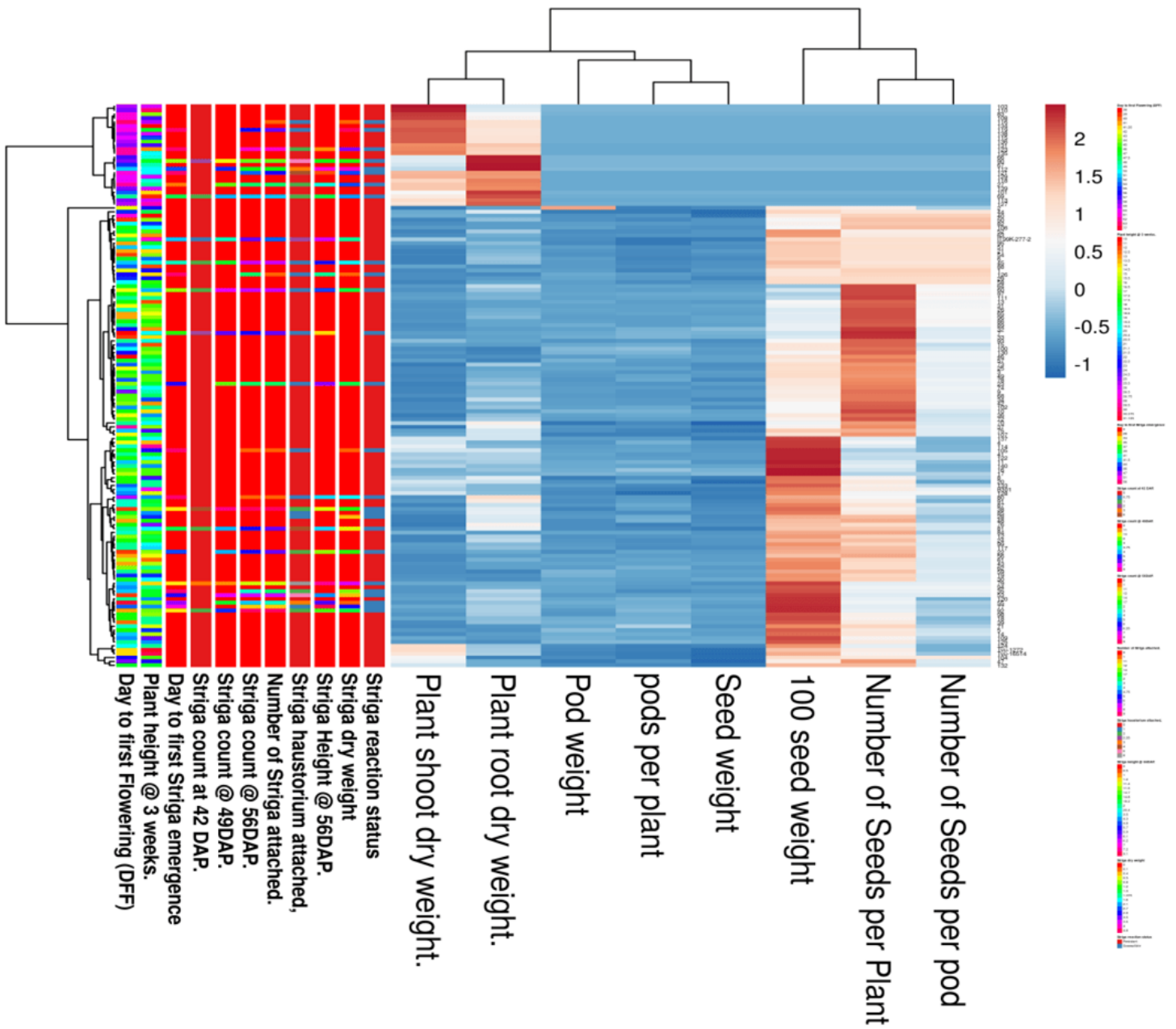


Figure 3

Hierarchical Cluster Dendrograms of BC_3F_2 Genotypes, Striga and Cowpea plant Parameters, and Heat map of Associations between Parameters.

Correlation Matrix - Population 1 and 2 on Selected Traits

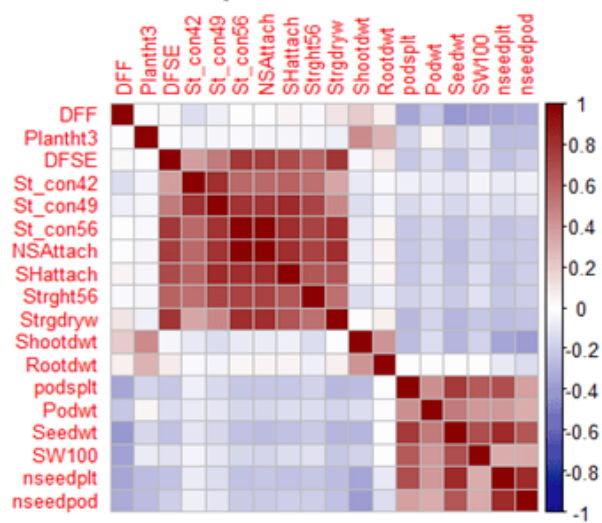


Figure 4

Heat Map of Correlation Matrix for Association Striga Parameters and Cowpea Plant Parameters in BC₃F₂ Population of Cowpea