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Identification of novel resistance sources to *Striga gesnerioides* in local Cowpea varieties through agronomic and molecular approaches

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ABSTRACT
Objective: To identify novel sources of resistance to *Striga gesnerioides* in local cowpea (*Vigna unguiculata*) varieties from Burkina Faso.
Methodology and results: Five local varieties (V1, V2, V3, V4, V6) and two improved varieties (V5, V7) were evaluated in pots to identify resistance sources against races 1 and KP. Agronomic performance was assessed using a randomized complete block design (RCBD), and molecular screening was conducted by PCR with the SSR1 marker following DNA extraction using the Whatman FTA card protocol. Molecular analysis confirmed the presence of resistance alleles to both races in the studied germplasm. Agronomic evaluation showed substantial genetic variability, particularly for grain yield (CV = 60.09%). Varieties V3, V5, and V7 combined resistance markers, high yield potential, and early maturity.
Conclusion and application of results: This study identified new sources of resistance to races 1 and KP of Striga gesnerioides in local cowpea varieties with the presence of 150 base pairs using the SSR1 marker. These genotypes constitute valuable resources for marker-assisted selection and potential direct release, contributing to reduced Striga-related yield losses and improved food security in Burkina Faso. These findings enable the direct use of this germplasm as an elite parental line in hybridization programs aimed at introgressing resistance to *Striga gesnerioïdes.*
Keywords: Cowpea, local varieties, *Striga gesnerioides,* molecular marker, SSR.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a grain legume widely cultivated in tropical and subtropical regions, particularly in sub-Saharan Africa. Annual global production is estimated at approximately 9.77 million tons, with Africa accounting for more than 95% of total production according to the latest FAOSTAT data (2024). In Burkina Faso, cowpea occupies a strategic position in both rural and urban areas. It represents a major source of plant protein for human consumption and an important fodder resource for livestock. Its cultivation also provides a significant source of income for many low-resource households. Despite its socio-economic and nutritional importance, cowpea production faces numerous biotic constraints. These include bacterial, viral, and fungal diseases, as well as various insect pests such as aphids, thrips, sap-sucking bugs, pod borers, and bruchids. In addition, parasitic weeds—particularly *Alectra vogelii* and *Striga gesnerioides*—pose serious threats. Among these, *S. gesnerioides* is one of the most destructive. It is a root hemiparasitic plant specific to cowpea, establishing a direct connection with the host root system. This close parasitic association severely disrupts water and nutrient uptake, resulting in stunted growth, plant wilting, and drastic yield reductions. Yield losses due to infestation can range from 83% to 100%, depending on infestation level and varietal susceptibility (Cardwell *et al.*, 1995; Asare *et al.*, 2013). Haruna *et al.* (2020) reported that the severity of damage caused by *Striga gesnerioides* is closely linked to the intimate host–parasite interaction. Several physiological races of *Striga gesnerioides* have been identified. Seven races were described by Lane *et al.* (1996), including race SG1, which has been reported in Burkina Faso. In addition, a new race designated KP was identified in the Koupéla region (Tignégré *et al.*, 2013). This racial variability further complicates parasite management and underscores the need for continuous identification of resistance sources adapted to local conditions. The use of resistant varieties remains one of the most effective, economical, and sustainable strategies for controlling *Striga gesnerioides*. However, currently available resistance sources do not always combine desirable agronomic and commercial traits such as high yield, early maturity, seed quality, and local adaptation. Moreover, limited studies have evaluated local cowpea varieties in Burkina Faso for resistance or tolerance to *Striga gesnerioides*, particularly using molecular biology tools. The use of molecular markers, such as the SSR1 marker, enables the detection of alleles associated with resistance to *Striga gesnerioides*, thereby accelerating varietal development for effective parasite control. In this context, the present study had a dual objective: to evaluate agronomic parameters and to determine the presence of resistance alleles to *Striga gesnerioides* in local cowpea varieties from Burkina Faso, with the aim of identifying genotypes potentially suitable for use in breeding programs.

MATERIAL AND METHODS

Plant material : The plant material used in this study consisted of five local varieties Bengringa flat brown hilum, Bengringa round brown hilum, Bengringa black hilum, Bengraga B, and Bengraga N and two improved varieties (Komcallé and IT98K-205-8), which possess multiple resistances to different races of *Striga gesnerioides*. The local varieties were sourced from the germplasm collection of the Oilseed and Protein Crop Program of INERA (Saria Research Station). Table 1 provides a detailed description of the plant material used in this study.

Table 1: Origin, cycle, and status of the cowpea varieties used in this study

Varieties	Abbreviation	Cycle	Status [1]	Origin
Bengringa plat hile brun	V1	96±5	Unknow	Burkina Faso
Bengraga B	V2	96±5	Unknow	Burkina Faso
Bengringa rond hile brun	V3	96±5	Unknow	Burkina Faso
Bengraga N	V4	96±5	Unknow	Burkina Faso
Komcallé	V5	60-65	Resistance to SG	Burkina Faso
Bengringa hile noir	V6	96±5	Unknow	Burkina Faso
IT98K-205-8	V7	60-65	Resistance to SG	Nigeria

[1] SG : *Striga gesnerioïdes*

Experimental site and trial conduct: The experiment was conducted at the Institute of Environment and Agricultural Research (INERA), at the Saria station (12.267° N, 2.150° W; 300 m above sea level), located in the North-Sudanian agro-ecological zone between the 700- and 900-mm isohyets, approximately 80 km from Ouagadougou. Pots were used to cultivate the seven cowpea varieties evaluated in this study, following a completely randomized block design. Each replication consisted of seven elementary plots spaced at 1 m x 0.5 m. Each plot contained four pots arranged in two rows, resulting in a total of 84 pots. Mineral fertilizers were applied at a rate of 100 kg ha^{-1} of NPK (14N–23P–14K–6S–1B) at 14 days after sowing.

Molecular characterization: The microsatellite marker or Simple Sequence Repeats (SSRs) has been used for the identification of *Striga gesnerioides* resistance gene. For this purpose, PCR (Polymerase Chain Reaction) reactions were run using a thermo cycler of the Eppendorf master cycler gradient 5332 version 2.30 marker. This marker has been used to detect the presence of the KP race Striga resistance gene (Omoigui *et al.* 2015, Larweh *et al.*, 2017; Sidibé *et al.*, 2021). The microsatellite or Simple Sequence Repeats (SSR) marker has the following sequence:

SSR1_Forward:
CAAGAAGGAGGCGAAGACTG
SSR1_Revers:
CCTAAGCTTTTCTCCAACTCC

Sampling: Leaf sampling consisted of selecting and collecting fresh young leaves from the cowpea varieties under study. The leaves were crushed using an appropriate pestle and Parafilm®, and the resulting plant sap was then carefully blotted onto FTA (Fast Technology for Analysis) cards for subsequent molecular analysis.

DNA extraction : For DNA extraction, 5 mm diameter discs were punched from each sample using a sterile puncher. The discs were placed into Eppendorf tubes, and 200 µL of 70% ethanol per disc was added. The discs were washed twice successively for 5 minutes each. After each washing step, the solution was carefully removed using a micropipette. The washed DNA discs were then solubilized in 200 µL of TE buffer (Tris–EDTA) per disc for 5 minutes, and this step was repeated twice successively. Following solubilization, the discs were spread out and air-dried at room temperature for 24 hours before being transferred into PCR tubes for amplification.

Polymerase Chain Reaction (PCR) amplification: PCR amplification was performed in a total reaction volume of 25 µL. For this purpose, a PCR master mix was prepared by adding 2 µL of premix (containing 1 U of Taq polymerase, 250 µM Tris-HCl, 10 mM KCl, and 1.5 mM $MgCl_2$) into each of the seven PCR tubes. Subsequently, 18 µL of ultrapure water was added to each tube, followed by the placement of the DNA discs extracted from the FTA cards containing the template DNA to be amplified.

Revelation of amplification products: For this step, it is important to prepare a 2% agarose gel supplemented with EtBr (ethidium bromide), a fluorescent dye. To prepare the gel, 2 g of agarose powder was weighed and mixed with 100 mL of TAE buffer (Tris-acetate-EDTA). The mixture was then heated in a suitable microwave oven for 5 minutes, and this heating step was repeated twice to ensure complete dissolution of the agarose. Once the agarose gel was properly prepared, the PCR products from the tubes were pipetted and loaded into the gel wells for electrophoresis. The migration was carried out for 1 hour. The amplified products were visualized under ultraviolet (UV) light and photographed for documentation.

Data collection: Six agronomic traits were measured during the trial to characterize the phenology, yield components, and productive performance of the genotypes. These included: the number of days to 50% flowering (FL50), used as an indicator of earliness; the number of days to 95% physiological maturity (CSPM); 100-seed weight (W100S) expressed in grams; total pod weight per plant (WOP); total grain weight per plant (Total Grain Weight); grain yield per hectare (GY); and the grain filling rate (FILR).

Data analysis : The data were analyzed using analysis of variance (ANOVA) based on a completely randomized block design conducted in a single environment. The linear model was implemented in R (R Core Team, 2024) through RStudio (Posit Team, 2025) as follows:

$Y_{ij} = \mu + R_i + G_j + \varepsilon_{ij}$

where Y_{ij} represents the observed value of genotype iii in replication j, μ is the overall mean, R is the fixed effect of replication j, and G is the fixed effect of genotype i

Broad-sense heritability (H^2) was estimated from the mean squares of the ANOVA according to the formula of Holland *et al.* (2002):

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_e^2}{n_R}}$$

Where σ_G^2 is the genetic variance, σ_e^2 is the residual variance, and n_R is the number of replications.

RESULTS

Phenotypic variation and heritability: Table 2 presents the means, genetic variances, and broad-sense heritability estimates of the studied traits. The mean values indicate substantial phenotypic variation among genotypes across all characters. Days to 50% flowering (FL50) and days to physiological maturity (CSPM) showed mean values of 48.23 and 69.8 days, respectively. Yield-related traits exhibited greater dispersion, with mean values of 14.09 g for 100-seed weight (W100S), 171.05 g for pod weight (WOP), 0.62 for filling rate (FILR), and 280.45 kg ha^{-1} for grain yield (GY). Genetic variances were highly significant for all traits ($p < 0.001$), with high broad-sense heritability estimates ranging from 0.84 to 0.98. The highest heritability values were observed for phenological traits (FL50 and CSPM, $H^2 = 0.98$), while yield-related traits also exhibited high heritability estimates (0.84 - 0.87).

Table 2: Mean, genetic variance and broad-sense heritability of agronomic traits

Traits	Mean ± SD	CV	σ^2_G	H^2
FL50	48.24±3.13	6.5	34.41***	0.98
CSPM	69.81±3.49	4.99	46.06***	0.98
W100S	14.09±4.07	28.89	22.57***	0.87
WOP	171.06±94.75	55.39	9810.85***	0.84
FILR	0.62±0.14	22.54	0.04***	0.93
GY	280.46±168.53	60.09	38580.71***	0.87

FL50: number of days at 50% flowering, CSPM: number of days at 95% maturity, W100S: seed weight per pod (100 seeds), WOP: total weight of pods, FILR: filling rate, GY: grains yield, SD: standard deviation, CV: coefficient of variation, σ^2_G: genetic variance, H^2: heritability in the broad sense, ***: $\alpha = 0.001$

Genotypic variation in mean performance: Significant differences were observed among varieties for mean performance in both phenological and yield-related traits. For phenological traits (Figure 1), days to 50% flowering (FL50) ranged from 43.17 days (V5) to 52.25 days (V2). Varieties V5 and V4 exhibited the shortest cycles, while V2 showed the longest. Days to physiological maturity (CSPM) varied from 63.83 days (V5) to 72.17 days (V1 and V6). Yield-related traits displayed a wider range of variation. 100-seed weight (W100S) ranged from 9.68 g (V4) to 16.99 g (V1), while pod filling rate (FILR) differed significantly among varieties, with the highest values observed in V5, V3, V6, and V7 (Figure 2). Pod weight (WOP) was highest in V3, V5, and V7, whereas V4 had the lowest value. Grain yield (GY) ranged from 106.06 kg ha^{-1} (V4) to 383.52 kg ha^{-1} (V3). The highest yields were observed in varieties V3, V5, and V7, which belong to the same statistical group (a), while V4 had the lowest yield (Figure 3).

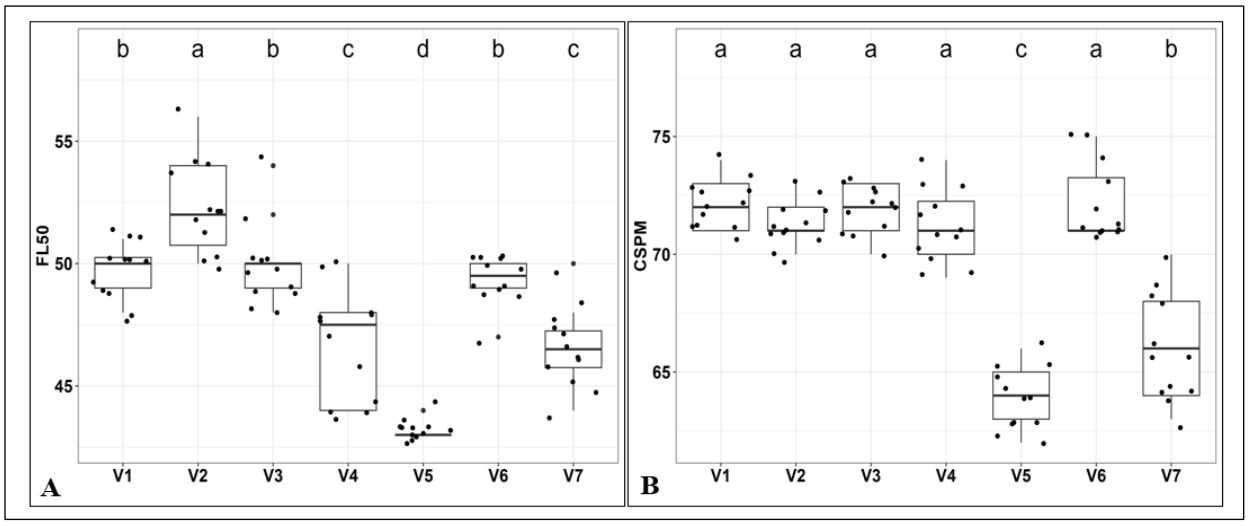

Figure 1: Variability of the phenological cycle of local and improved varieties (A: FL50, B: CSPM)

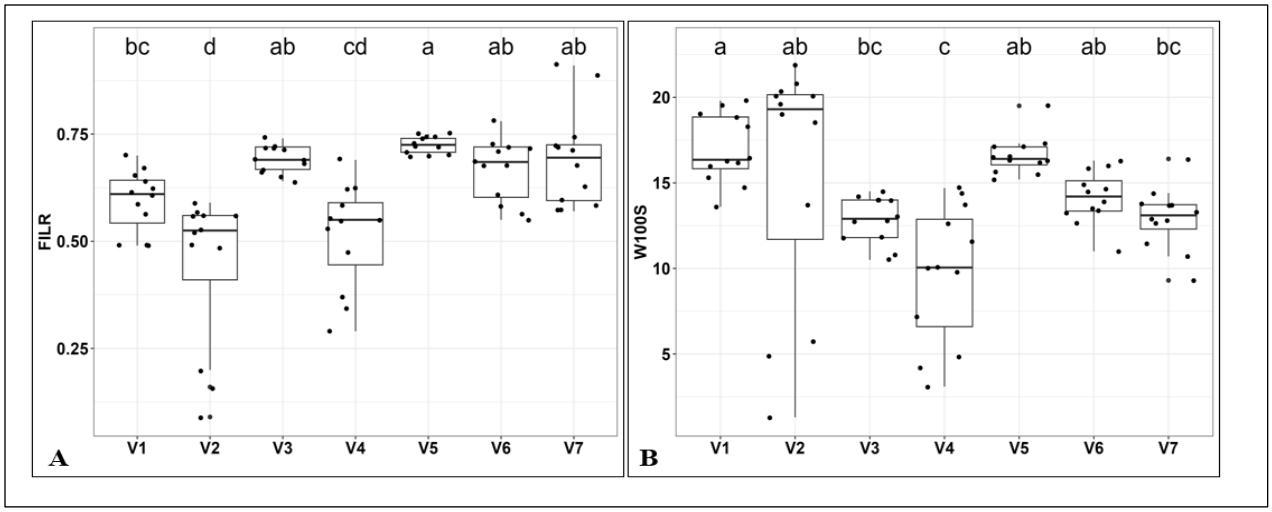

Figure 2: Genetic variation in grain filling (A: FILR) and 100-grain weight of local and improved varieties (B: W100S)

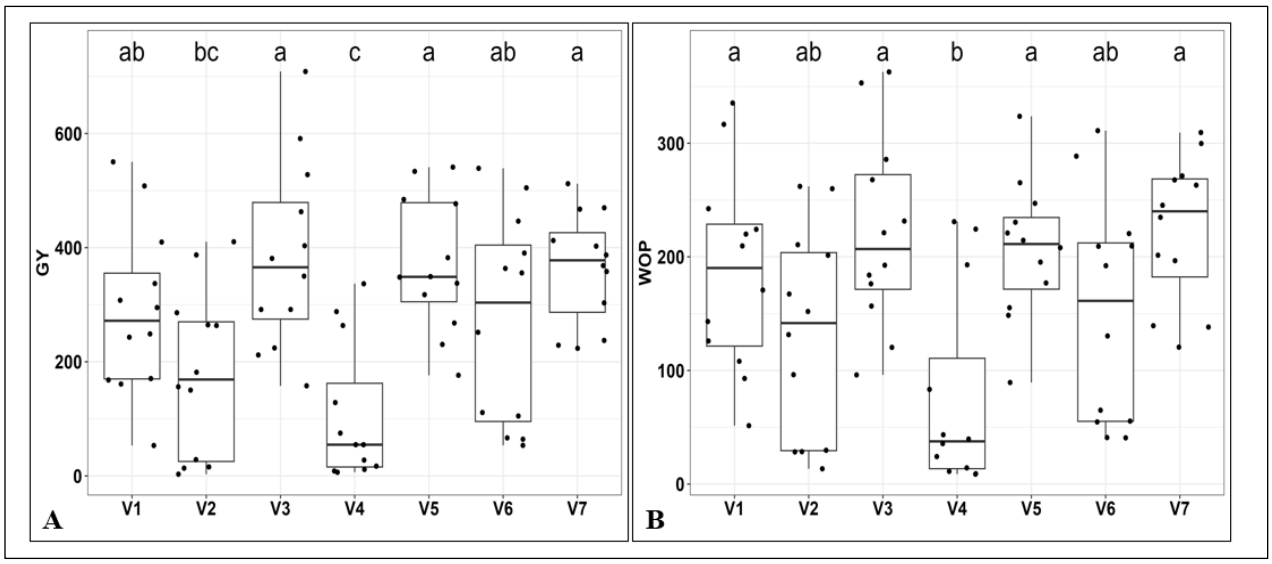

Figure 3: Genetic variation in grain yield (A: GY) and total pod weight per plant (B: WOP)

Principal component analysis and correlation between traits: The principal component analysis (PCA) performed on the six traits explained 75.2% of the total variation on the first two axes, with 52.9% for Dim1 and 22.3% for Dim2 (Figure 4A). Dim1 was primarily associated with yield-related traits, including pod weight (WOP), grain yield (GY), grain filling rate (FILR), and 100 seed weight (W100S), whereas the phenological traits FL50 and CSPM were associated with Dim2. Additionally, the correlation matrix confirmed the relationships highlighted by the PCA (Figure 4B). A strong positive correlation was observed between FL50 and CSPM, indicating coherence between flowering and maturity. Yield and filling traits exhibited high positive correlations among themselves, particularly between WOP and GY, as well as between GY and FILR. In contrast, FL50 and CSPM were negatively correlated with GY, WOP, and FILR.

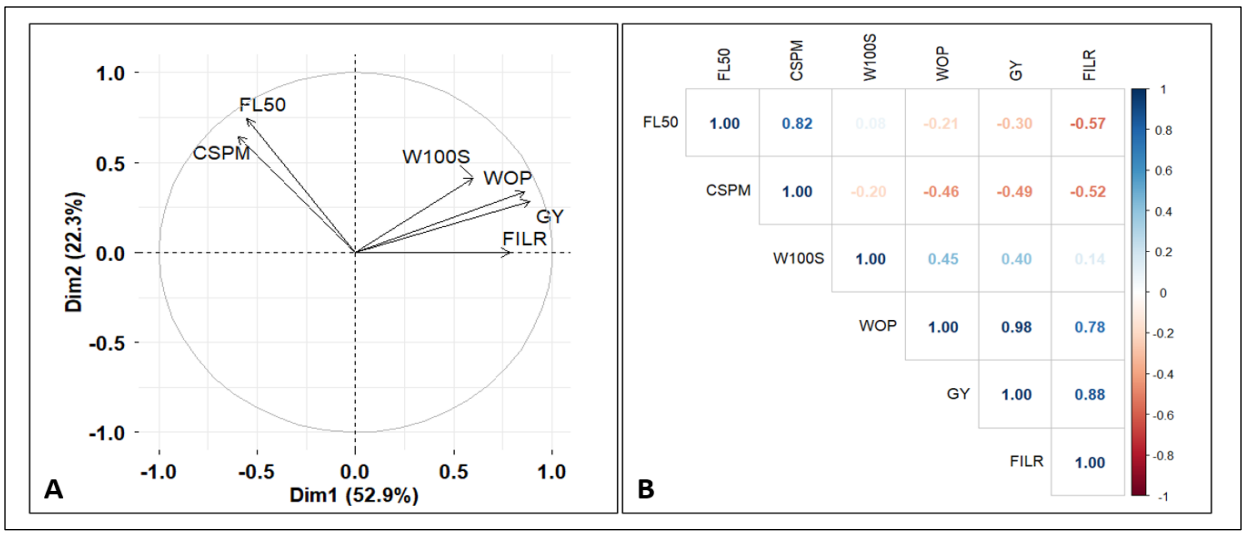

Figure 4: Principal component analysis and correlation between phenology and agronomic traits. A: Location of variables on the first two axes of the PCA, B: Correlation between traits.

Identification of resistance alleles to *Striga gesnerioides* using the SSR marker: The results of the Striga gesnerioides resistance analysis showed that the five local cowpea varieties (Bengringa plat hile brun, Bengringa rond hile brun, Bengraga N, Bengraga B, Bengringa hile noir) as well as the control varieties (IT98K-205-8 and Komcallé) are resistant to Striga gesnerioides. Indeed, based on the gel profiles, all seven varieties displayed the resistance band at 150 bp. The photograph below provides an example of a gel profile (Figure 5).

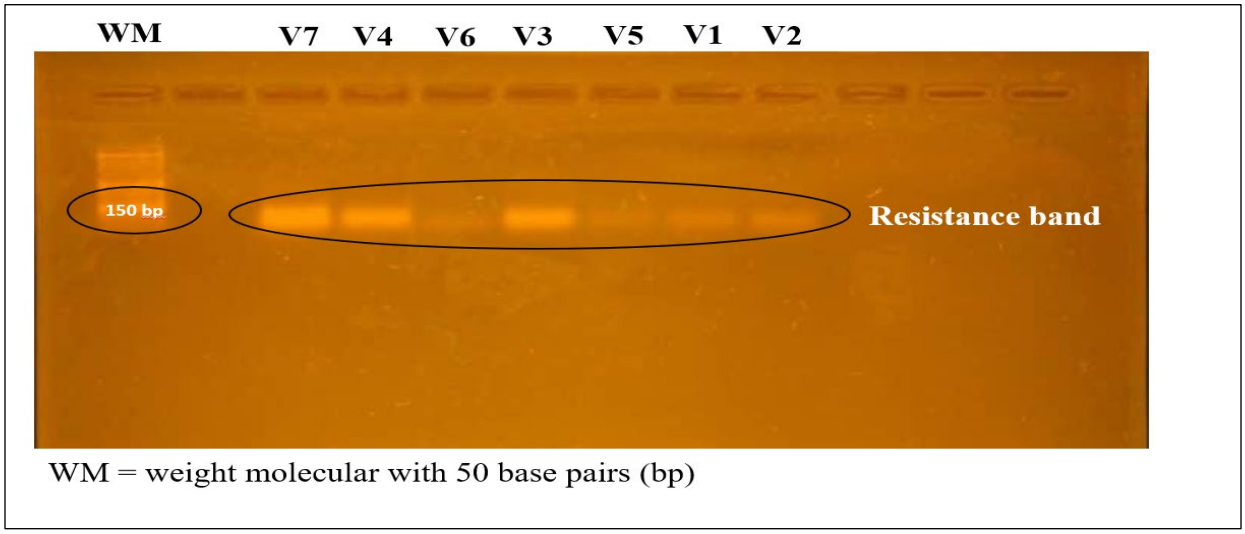

Figure 5: Identification of resistance to *Striga gesnerioides* by PCR amplification of DNA extracts from seven cowpea varieties.

DISCUSSION

The combined evaluation of agronomic traits and the presence of alleles associated with resistance to *Striga gesnerioides* provides a better understanding of the adaptability of local varieties under parasite pressure. Moreover, local varieties represent a valuable reservoir of genetic diversity, which can be exploited for the identification and introgression of resistance or tolerance alleles into breeding programs. The results of this study contribute to the identification of potential sources of *Striga gesnerioides* resistance while taking agronomic performance into account.

Agronomic evaluation of local and improved varieties and the relationships among traits: The data analysis revealed a very high and significant variability, with strong selection potential for all traits studied (Figures 1, 2, and 3). This presence of significant variation among the varieties for all traits indicates substantial exploitable genetic diversity. According to Robinson (1966), heritability is classified into three categories: low (0–0.30), moderate (0.30–0.60), and high (>0.60). The heritability values observed in this study exceeded 0.80, indicating that environmental influence remained limited during the trial. As noted by Dabholkar (1992), this allows for effective selection based solely on the phenotype of individuals. The high variability observed, particularly for grain yield (GY, CV = 60.09%), suggests a considerable level of genetic diversity within the material studied. According to Shiva *et al.* (2013), a high genetic coefficient of variation is an indicator of a population's allelic richness, which is a prerequisite for any genetic improvement. Varieties V3, V5, and V7 exhibited the highest grain yields (GY of 383.52a, 370.48a, and 364.28a, respectively). These superior performances often result from a favourable combination of traits. The strong positive correlation observed between pod weight (WOP) and grain yield indicates that WOP is a reliable indirect selection criterion for improving GY. Regarding the growth cycle, the earliness observed in improved varieties V5 and V7, particularly the time from sowing to 50% flowering (FL50) of approximately 43–46 days, can be a major advantage for adaptation to stressful environments such as drought, allowing plants to escape certain climatic constraints. These varieties therefore constitute a potential source for introgression to improve the cycle of local varieties, which exhibit intermediate to late cycles (Figure 1). Additionally, the pod filling rate, which is correlated with GY and observed in local varieties V3 and V6 belonging to the same group as improved varieties V5 and V7, indicates that this trait is a good predictor of higher production and consequent yield increases in cowpea cultivation. This also highlights the potential of the local varieties used. Since this study was conducted in pots, these results confirm the need to evaluate the genetic material under field conditions to identify the most productive and locally adapted varieties, as recommended by Hikmat *et al.* (2012) regarding the importance of participatory varietal trials.

Identification of resistance alleles to *Striga gesnerioides* in local varieties: The results revealed that the SSR1 marker was able to identify the resistant genotypes among the five evaluated local varieties. Previous studies have shown that this marker is associated with resistance to *Striga* race 1 (Ouédraogo *et al.*, 2012; Asare *et al.*, 2010) and to races 1 and KP (Sidibé *et al.*, 2021). Molecular analyses indicated that the evaluated genotypes produced a 150-base pair (bp) amplification product, corresponding to the expected profile for the marker linked to resistance against *Striga gesnerioides* races 1 and KP. The presence of this specific band confirms the existence of at least one resistance allele within the genetic makeup of the five tested genotypes. These results are consistent with

molecular mapping studies (Ouedraogo *et al.*, 2001; Li and Timko, 2009), which identified the SSR1 microsatellite marker as closely linked to resistance genes against SG3 and SG5 races as well. Indeed, it is scientifically established that genes conferring resistance to SG3 and SG5 parasite races are mapped on chromosome Vu11 (linkage group 1) of cowpea (Ouedraogo *et al.*, 2001; Omoigui *et al.*, 2016). These findings align with the work of Omoigui *et al.* (2009), who demonstrated the effectiveness of this marker in identifying resistant lines via a similar amplification band. The detection of this marker in all evaluated genotypes suggests that they possess the genetic determinants necessary to counter *S. gesnerioides* infestation. Furthermore, this study highlights the limitations of visual field evaluation. As noted by Omoigui *et al.* (2015), selection of genotypes cannot rely solely on phenotypic data, which are often influenced by environmental variation and heterogeneous parasite pressure. The use of molecular markers, notably SSRs (Simple Sequence Repeats) and SCARs (Sequence Characterized Amplified Regions), provides a more reliable and precise strategy to distinguish truly resistant genotypes from susceptible ones (Omoigui *et al.*, 2015). The integration of marker-assisted selection thus secures the breeding process by ensuring the presence of target genes, independently of field screening conditions.

CONCLUSION AND APPLICATION OF RESULTS

The results of this study highlighted the presence of resistance alleles to SG1 and KP races associated with favourable agronomic traits, including grain yield, pod yield, and earliness, in both improved and local varieties. Among the local varieties, V3 stood out for its good yield potential and earliness, indicating an interesting combination of local adaptation and genetic resistance. Similar to the improved varieties V5 and V7, V3 could therefore serve as a potential elite parent in hybridization programs aimed at introgressing *Striga gesnerioides* resistance genes. These findings underscore the strategic importance of utilizing local varieties as sources of exploitable genetic diversity for the development of high-performing cultivars that are sustainably resistant to the dominant parasite races in Burkina Faso. The resistant local varieties identified in this study should be prioritized as donor parents in cowpea improvement programs. Their integration into marker-assisted and genomic breeding schemes, combined with resistance gene pyramiding and multi-environment evaluation, will facilitate the development of high-yielding cultivars with durable resistance to the predominant *Striga gesnerioides* races in Burkina Faso. Continuous monitoring of parasite race diversity and the conservation of local germplasm resources will be essential to sustain long-term breeding gains.

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